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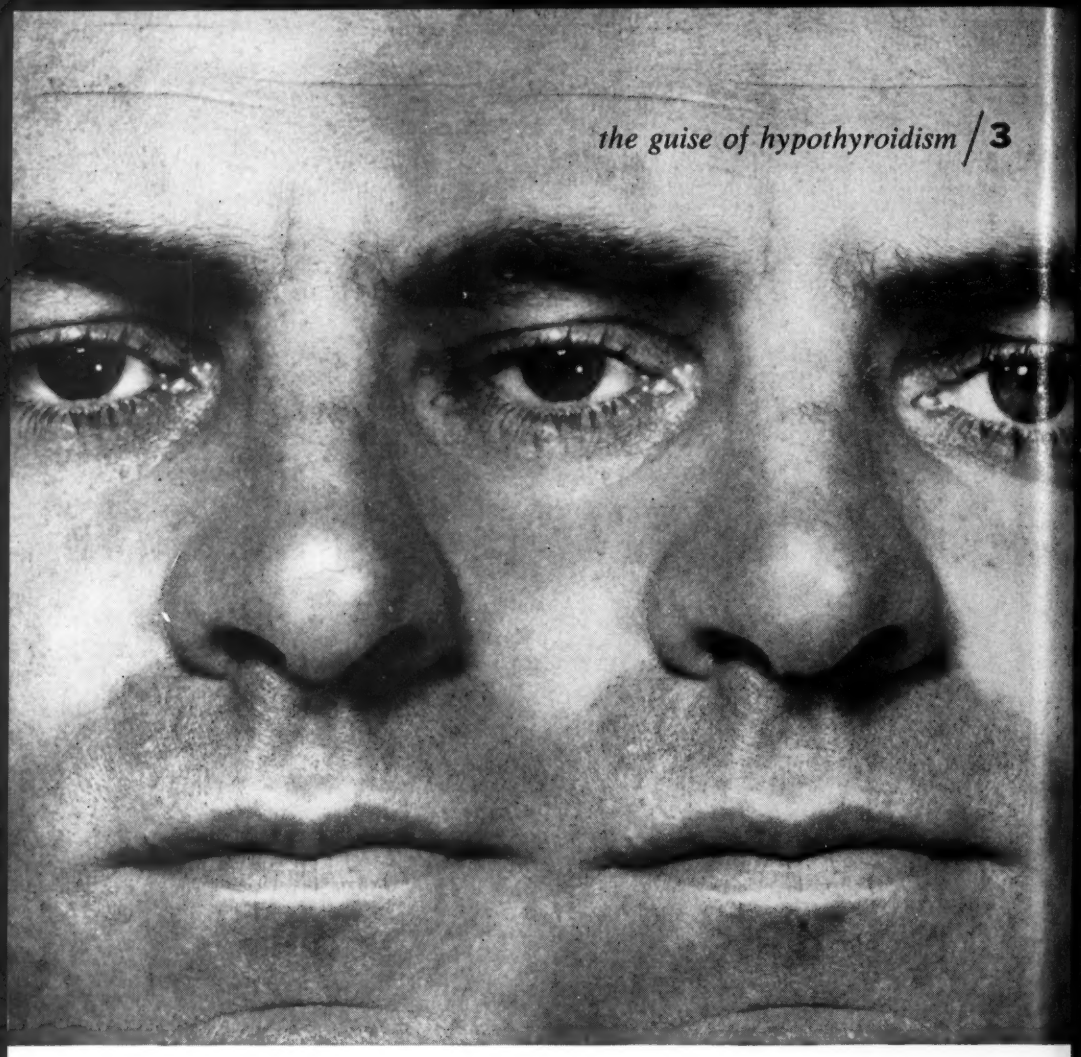

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EFFECTS OF HOG PITUITARY FOLLICLE-STIMULATING HORMONE IN WOMEN: ANTIHORMONE FORMATION AND INHIBITION OF OVARIAN FUNCTION*

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IN ADDITION to their hormonal actions, gonadotropins may evoke the formation of antihormones. This phenomenon, which is similar if not identical to antibody formation, is seen when a gonadotropin derived from one species is administered for a prolonged period to an individual of another species (1). In a previous study (2) antihormones were detected by the second month of therapy in each of 7 infertile men who received daily injections of sheep pituitary follicle-stimulating hormone (FSH). The antihormones prevented the actions of several gonadotropins, including human pituitary gonadotropin. Neutralization of the patients' endogenous gonadotropins resulted in inhibition of spermatogenesis (3). The number

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of sperm in the seminal fluid fell below pretreatment levels, and testicular morphology, studied by repeated biopsies, regressed to a state resembling that seen after hypophysectomy.

Therefore, when FSH prepared from hog pituitary glands was made available to us, the problem of antihormone formation was studied, as well as the stimulatory effects of the preparation.

CLINICAL MATERIAL

Hog pituitary FSH was administered to 9 female patients. Urinary estrogen excretion was measured as the criterion of ovarian response. In addition, ovarian and endometrial biopsies and urinary pregnanediol studies were performed in a few patients.

Antihormone studies were carried out in 4 patients who received FSH for periods of two months or longer. Patient M.S. was an 18-year-old girl having sickle-cell anemia and hypogonadism associated with low urinary gonadotropin excretion. Puberal development was first noticed at the age of 13 years. She had a few menstrual periods when she was 15 years of age, but none thereafter. Skeletal proportions were eunuchoidal and sexual development had not progressed beyond the stage of slight breast development and sparse pubic hair growth. FSH therapy was given to stimulate ovarian estrogen secretion with the hope that function would continue after treatment. Patient L.S., a 42-year-old women who had previously undergone unilateral ovariectomy, volunteered to continue with the studies after an initial course of FSH which had been given to evaluate ovarian function.

The other 2 patients in this group of 4 were individuals in whom temporary cessation of ovarian function was considered desirable. FSH was administered to induce formation of antihormones, the object being to inhibit ovarian function by neutralizing endogenous gonadotropins. Patient B.F. was a 7-year-old girl having constitutional precocious puberty. This diagnosis was based on a history of regular menses for eight months, breast development beginning at the age of 6 years, pubic and axillary hair growth, urinary gonadotropin and estrogen excretion within the range for normal adult women, absence of signs of intracranial or pelvic abnormality, and normal bones by x-ray examination except for advanced epiphyseal development approximating that of a 10-year-old child. Patient M.C. was a normally developed 18-year-old girl who had a four-year history of severe functional uterine bleeding. She had received various forms of hormonal therapy and had undergone uterine curettage on several occasions.

Five patients received FSH for periods of three weeks or less. Three of these patients underwent hysterectomy for uterine fibroids, at which time

ovarian biopsy specimens were obtained. The other 2 had undergone bilateral ovariectomy prior to receiving FSH.

PREPARATIONS AND METHODS

FSH preparations. Two lots of hog FSH[1] were used. Lot R377200 contained 1.0 unit per mg. of dry powder, and the manufacturer's assay showed very small amounts of thyrotropin (TSH) and interstitial-cell stimulating hormone (ICSH) and negligible quantities of corticotropin (ACTH), growth hormone and prolactin. Lot R527090, a more purified preparation, contained 5 units per mg. of dry powder. "Units" refers to the labelled potency, which was determined by the method of Steelman *et al.* (4). Seven patients received Lot R377200; Patient M.S. received Lot R527090; and Patient R.W. received a commercially available sheep pituitary FSH preparation.[2]

Frequent assays were performed in our laboratory to check stability of the preparations. The potency of the dry powder stored at 4° C. remained constant for two years. Once dissolved in water, however, potency was maintained at 4° C. for only two weeks (Table 1). Therefore, it was our policy to use the hormone within two weeks after dilution.

TABLE 1. STABILITY OF HOG FSH STORED IN AQUEOUS SOLUTION AT 4° C.

Days stored before starting assay	Units* per rat	Uterine weight (mg.)	Ovarian weight (mg.)	Number of rats
0–7	0.5	119	28.8	7
14	0.5	132	24.5	4
21	0.5	104	18.3	5
28–42	0.5	48	15.6	5
	0 (controls)	30	10.3	19

* Dissolved in 6 cc. of water; 1 cc. injected twice daily for three days and rats killed twenty-four hours after the last injection.

Urinary estrogens were assayed as previously described (5). In this method, the sodium hydroxide-soluble fraction of the chloroform extract of acid-hydrolyzed urine is administered to immature female rats. Increases in uterine weight induced by graded doses of the urine extract are compared with responses induced by estradiol benzoate and the results are expressed as micrograms per 24-hour urine specimen.

Urinary pregnanediol was determined by the method of Somerville *et al.* (6).

Plasma antihormone levels were assayed by determining the ability of the patient's plasma to inhibit the action of gonadotropins administered to rats. A representative assay is presented in Table 2. In any one assay, all rats receive the same amount of hormone. One group of rats receive only gonadotropin; others receive in addition various amounts of the patient's plasma. Usually two or more levels must be tested in order to determine the least amount of plasma that will inhibit the administered gonadotropin.

Initially, hog FSH was used as the "test hormone" in the antihormone assays. The

[1] Generously supplied by The Armour Laboratories, Chicago.

[2] "Gonadophysin," G. D. Searle & Co.

TABLE 2. ANTIHORMONE ASSAY OF PATIENT M.C.'S PLASMA OBTAINED SIX DAYS AFTER RECEIVING HOG FSH, 25 UNITS TWICE DAILY FOR NINETY DAYS

TEST HORMONE*	PLASMA† (cc. per rat)	ASSAY RATS‡			ANTIHORMONES§ (units in estim'd tot. plasma vol.)
		Uterine wt. (mg.)	Ovarian wt. (mg.)	No. of rats	
Hog FSH	—	94	16.8	6	500
(0.5 unit	1.0	114	20.4	3	
per rat)	3.0	34	9.9	3	
Human CG	—	142	14.3	6	
(2.0 units	0.1	135	13.8	3	30,000
per rat)	0.2	28	10.4	3	
None (controls)		31	10.4	9	

* Dissolved in 3.0 cc. of water; 0.5 cc. injected twice daily for three days.

† Diluted to 3.0 cc.; 0.5 cc. injected twice daily for three days. Plasma and hormone injected concomitantly at separate subcutaneous sites.

‡ Sprague-Dawley rats, 25 days old at the time of the first injection.

§ Plasma volume estimated at 3,000 cc.

Note that in this representative example, 3.0 cc. was the amount of plasma needed to prevent the action of 0.5 unit of hog FSH, whereas 0.2 cc. of the same plasma inhibited the action of 2.0 units of CG. Thus, for a given plasma sample, antihormone titers expressed as "anti-CG units" are approximately 50 to 100 times greater than when hog FSH is used as the test hormone.

antihormone activity of the patient's plasma was also tested against human chorionic gonadotropin (CG), human pituitary gonadotropin and rat pituitary gonadotropin. When CG was used instead of hog FSH as the "test hormone" only one tenth to one twentieth as much plasma was needed to demonstrate antihormone activity, This offered a more sensitive method for detecting antihormones and therefore was the method employed in the patients most recently studied (Patients M.S. and L.S.; Figs. 6 and 7). A discussion of the mechanisms accounting for the increased sensitivity of the "CG method" and a comparison of it with the conventional method of assay are presented in another publication (7).

Plasma antihormone levels are expressed in Figures 4 through 7 as "units in estimated total plasma volume." A unit of antihormone is defined as that amount that will just neutralize 1 unit of the test hormone. Plasma volume is estimated as 5 per cent of body weight. An example of these calculations is presented in the legend to Table 2.

RESULTS

Initial effects of FSH

Urinary estrogen excretion. Before treatment, urinary estrogen levels were normal in 6 patients and low in Patient M.S. and the 2 castrated

women (Table 3). Urinary estrogen excretion increased during FSH administration in each of the 7 patients having ovaries, but not in the 2 castrated women. The increase was evident by the first week and reached a peak during the second or third week of FSH treatment.

Post-treatment studies were performed in Patients R.W. and S.A., who underwent hysterectomy at the conclusion of 8-day and 16-day courses of FSH. Urinary estrogens were low a week after stopping FSH and then

TABLE 3. SUMMARY OF CHANGES IN ESTROGEN EXCRETION DURING FIRST MONTH OF FSH THERAPY

Patient	Age (yrs.)	Diagnosis	FSH therapy		Average urinary estrogen excretion§ (micrograms per 24 hrs.)			
			Type *	Tot. units per day†	Before therapy	First week of therapy	Second week of therapy	Third week of therapy
S.A.	29	Uterine myomata	Hog A	50	1.2	5.4	26.7	36.0
R.W.	29	Uterine myomata	Sheep‡	100	1.6	5.3	9.8	
S.E.	30	Uterine myomata	Hog A	50, 25	1.9	5.0	4.5	
B.F.	7	Constitutional precocious puberty	Hog A	25	0.8		5.1	
M.C.	18	"Functional bleeding"	Hog A	50	2.8		32.3	163.8
L.S.	42	Unilateral oophorectomy	Hog A	50	2.7	4.7	24.8	15.0
M.S.	16	Sickle-cell anemia with hypogonadism	Hog B	50	<0.2	0.3	1.0	2.0
C.B.	36	Castrated 4 yrs. earlier	Hog A	50	0.2	0.4	<0.2	<0.2
C.T.	48	Castrated 1 yr. earlier	Hog A	50	0.2		0.2	

* "Hog A" is lot R377200; "Hog B" is lot R527090.

† Divided into two equal daily doses.

‡ "Gonadophysin," G. D. Searle & Co.

§ Our range of normal values for 19 women of reproductive age is 0.9 to 4.5 micrograms per 24 hours, with an average of 2.4 micrograms.

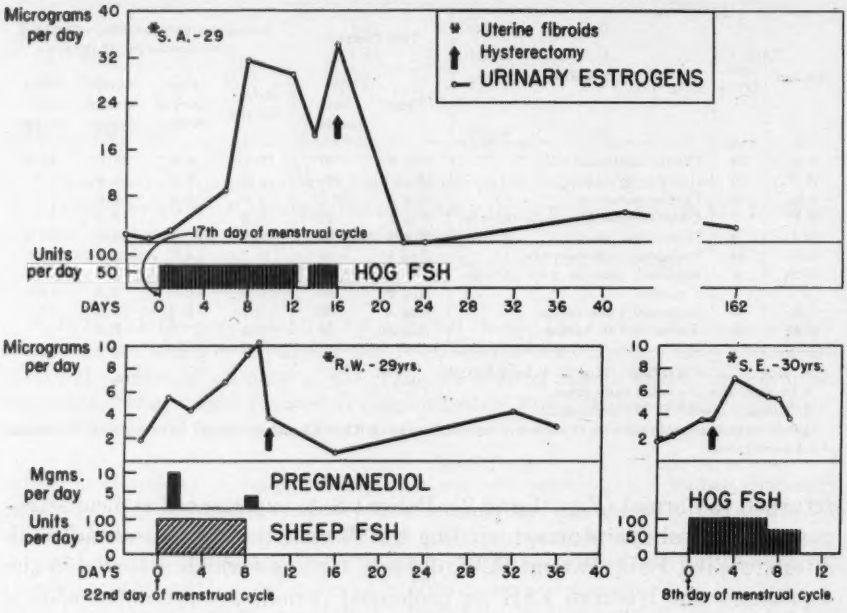
returned to normal (Figs. 1 and 2). Patient S.A. experienced typical estrogen withdrawal symptoms, including hot flashes, during the second week after stopping FSH; Patient R.W. did not. Changes which occurred in the 4 patients who received FSH for prolonged periods are presented after a description of the antihormone studies.

Breast changes. During the second or third week of treatment, 4 patients noticed fullness of the breasts and tenderness of the nipples. In Patient S.A. breast changes rapidly disappeared after stopping a 16-day course of FSH, whereas in Patients M.C., M.S. and L.S. who received FSH for prolonged periods, breast changes gradually disappeared during the second or third month of treatment. In Patient M.S., the hypogonadal girl, there was a definite increase in mammary tissue, which was maintained after stopping treatment.

Ovarian changes. Enlarged cystic ovaries, measuring 7 to 10 cm. in greatest dimension, were seen at laparotomy in Patients R.W. and S.A. The surfaces were covered with clear cysts as large as 2 cm. in diameter, which

microscopically were lined with granulosa cells undergoing early lutein changes. A 10-cc. portion of clear cyst fluid was withdrawn from the right ovary of Patient S.A. The estrogen content of this fluid, assayed in the manner described for urine extracts, was equivalent in biologic activity to 79 micrograms of estradiol benzoate per 100 cc. Ovarian biopsy specimens obtained from Patient S.E. on the second day of FSH administration were unremarkable except for evidence of old pelvic inflammatory disease and a paucity of follicles.

Laparotomy was not performed in the other patients but pelvic examina-



FIGURES 1, 2, 3

tions disclosed changes indicative of ovarian enlargement in Patients L.S., M.S. and M.C. In Patient L.S., a left adnexal mass about twice the size of a normal ovary was first palpated during the second month of FSH treatment. Two months after treatment this no longer could be felt. In Patient M.S. a similar mass was felt after one month of treatment. This was no longer palpable two weeks after stopping FSH. In Patient M.C., bilateral masses which could be felt on abdominal as well as pelvic examination, appeared during the second month of treatment. The greatest dimension of these masses, which were about equal in size, was estimated

as 15 cm. They gradually decreased in size and at the end of three months' treatment no longer were palpable.

Endometrial changes were studied in 3 patients. In Patient S.A. early secretory changes were present in a specimen of endometrium obtained the day FSH was started, seventeen days after the onset of the last menstrual period. On the tenth day of FSH treatment, early proliferative changes were seen, and on the sixteenth and last day, when hysterectomy

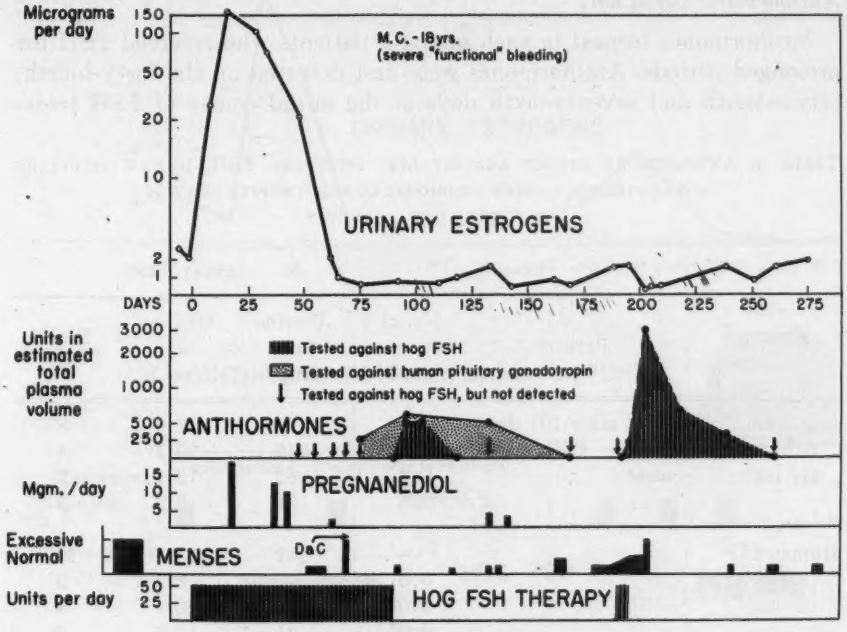


FIGURE 4

was performed, secretory changes were present. Patient R.W. received FSH from the twenty-second to the thirtieth day of the menstrual cycle. Secretory endometrium was present in the fibroid uterus removed the day after stopping FSH. In Patient M.C. uterine curettage had been performed on several occasions during the preceding four years for severe functional bleeding, and examinations of the tissue had revealed hyperplastic proliferative endometrium. FSH therapy was started two weeks after her most recent episode of menorrhagia. Endometrial biopsies were interpreted as follows: on the fifteenth day of FSH administration, "early secretory changes"; on the twenty-seventh and forty-first days, "marked secretory

changes and stromal edema reminiscent of a decidual reaction"; on the seventieth day, "proliferative endometrium"; and on the ninetieth and last day of treatment, "resting endometrium."

Urinary pregnanediol excretion was determined on a few occasions in Patients R.W. and M.C. (Figs. 2 and 4). The levels were correlated with the microscopic appearance of the endometrium, values of 5 mg. or more being associated with secretory endometrial changes.

Antihormone formation

Antihormones formed in each of the 4 patients who received FSH for prolonged periods. Antihormones were first detected on the forty-fourth, fifty-seventh and seventy-sixth days of the initial course of FSH treat-

TABLE 4. ANTIHORMONE EFFECT AGAINST HOG PITUITARY FSH, HUMAN PITUITARY GONADOTROPIN, HUMAN CHORIONIC GONADOTROPIN, AND RAT PITUITARY GONADOTROPIN

TEST HORMONE	PLASMA		ASSAY RATS		
	Patient	No. of cc. per rat	Uterine wt. (mg.)	Ovarian wt. (mg.)	No. of rats
Hog FSH (0.5 unit per rat)	B.F., after 101 days of hog FSH treatment	—	91	17.0	8
		0.05	119	29.7	4
		0.2	82	12.1	3
		0.5	21	9.3	3
Human CG (2.0 units per rat)		—	117	13.6	10
		0.01	127	8.3	3
		0.02	60	10.3	4
		0.05	31	10.5	5
Human APG*		—	114	50.2	3
		3.0	98	19.9	3
Rat APG†	M.C., after 148 days of hog FSH treatment	—	93	33.9	4
		0.10	100	15.2	3
		0.25	25	10.3	3

* Human anterior pituitary gonadotropin, concentrated from the urine of postmenopausal women by ultrafiltration. One-twelfth of the concentrate from a 24-hour urine specimen was administered to each rat.

† Rat anterior pituitary gonadotropin. The anterior pituitaries of immature rats were suspended in water by repeatedly drawing the glands into, and expelling them from a syringe. A total dose equivalent to 2 anterior pituitary glands was administered to each rat.

ment in Patients B.F., M.S. and M.C., respectively. In Patient L.S. antihormones were first detected on the tenth day of a second course begun two months after an initial 42-day course of FSH treatment (Fig. 6). The antihormones prevented the action of each of the gonadotropins against which they were tested, namely, hog FSH, human pituitary gonadotropin, human chorionic gonadotropin[3] and rat pituitary gonadotropin. An example is shown in Table 4. Tables listing results of the antihormone assays

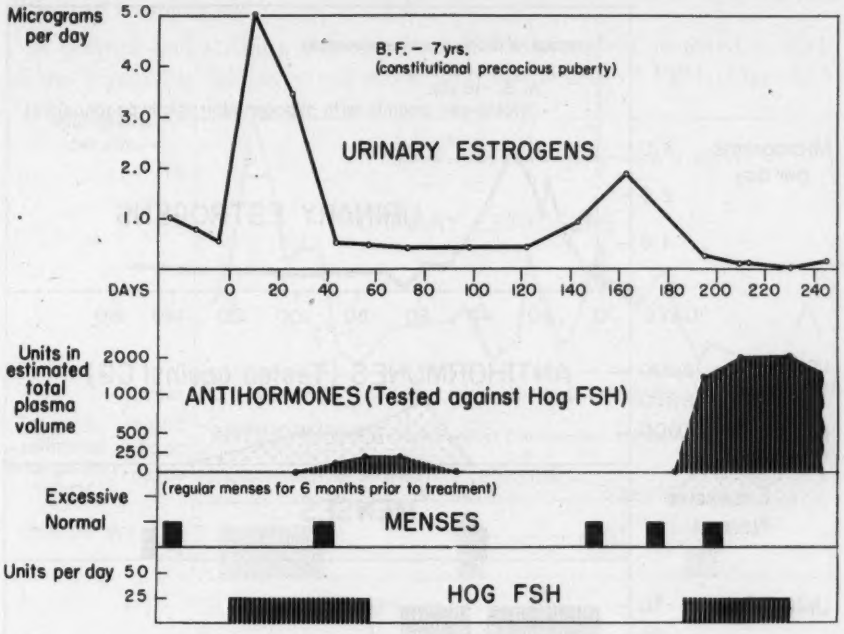


FIGURE 5

for each patient and a detailed discussion of the antihormone problem will be presented in another publication (8).

Effects of antihormones on ovarian function

The responses of the 3 patients who formed antihormones during the initial course of FSH treatment were similar (Figs. 4 to 6). Urinary estro-

[3] Both "A.P.L." and "International Standard for Chorionic Gonadotrophin" were used. A.P.L. was supplied by Ayerst, McKenna and Harrison, Ltd., and the International Standard was obtained from the U.S.P. Reference Standards. The antihormones have no direct neutralizing action against chorionic gonadotropin. Their ability to prevent the action of this hormone in female rats is a result of their ability to neutralize rat pituitary gonadotropin (7).

gen excretion, which had increased during the first month of FSH administration, fell during the second or third month to less than normal levels. The decrease in urinary estrogens coincided with, or shortly preceded the initial detection of antihormones. Coincident with the fall in urinary estrogen levels, each of the patients experienced vaginal bleeding.

Antihormones remained at detectable levels for two to three months after stopping FSH. In Patient M.C., urinary estrogens remained at or

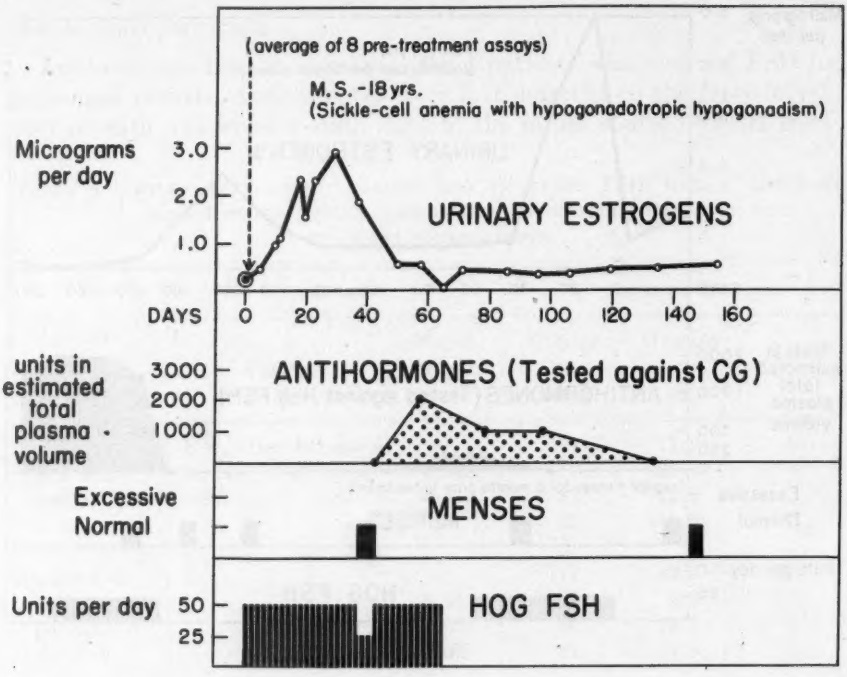


FIGURE 6

below the lower limits of normal for three months after treatment (Fig. 4). During this period no vaginal bleeding occurred, except for a few episodes of "spotting." She then had a menstrual period of normal amount and duration. This was followed by a prolonged episode of bleeding, at which time a second course of FSH was given. Patient B.F. excreted subnormal amounts of estrogens and did not menstruate for three months after stopping FSH. Urinary estrogens then increased and menses recurred. In Patient M.S., estrogen excretion remained at lower than normal levels for the three months during which post-treatment determinations were performed. Three months after stopping FSH she experienced a normal

menstrual period. She received no further treatment and during the ensuing eight months she had fairly regular menses.

Antihormones were not detected in Patient L.S. during an initial 42-day course of FSH. Urinary estrogen levels remained elevated throughout treatment and fell to low levels shortly after stopping FSH. In contrast to the patients who formed antihormones, estrogen excretion returned to normal within a month after discontinuing FSH.

Effects of a subsequent course of FSH

A prompt and striking increase in antihormone titers occurred in each of the 3 patients who received more than one course of FSH (Figs. 4, 5

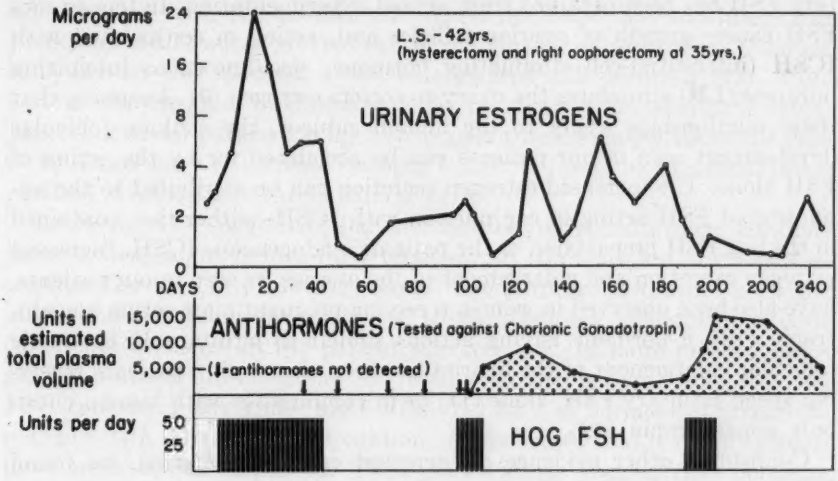


FIGURE 7

and 7). The responses of Patients M.C. and B.F. were similar. Within two weeks antihormone titers reached levels several times those observed during the initial course of FSH. Urinary estrogen levels, rather than increasing as they did during the initial period of treatment, fell to low or undetectable levels. During the week following the second course of FSH, the period when estrogen levels were lowest, Patient M.C. experienced hot flashes. These occurred several times a day, were most noticeable in the face, and each episode lasted two or three minutes. A bout of prolonged vaginal bleeding ceased two weeks after starting the second course of FSH and during the next three months only occasional vaginal spotting occurred. Patient B.F. had a normal menstrual period beginning eight days after starting the second course of treatment. A two-month period of amenorrhea then ensued.

In Patient L.S., antihormones were first detected on the tenth and last day of the second course of FSH. Estrogen excretion was normal before, and did not change during the ten days FSH was given. Except for one low value observed the week after stopping FSH, estrogen excretion remained within the normal range for the next two and a half months. A third course of FSH resulted in a rapid increase in antihormone titers and a decrease in estrogen excretion to subnormal levels. These changes persisted for approximately one month.

DISCUSSION

Most of the information concerning the gonadotropic actions of pituitary FSH has been obtained from animal experimentation. In female rats FSH causes growth of ovarian follicles and, acting in conjunction with ICSH (interstitial-cell stimulating hormone, also known as luteinizing hormone, LH) stimulates the ovary to secrete estrogen (9). Assuming that these relationships apply to the human subject, the striking follicular development seen in our patients can be accounted for by the action of FSH alone. The increased estrogen secretion can be attributed to the administered FSH acting in conjunction with ICSH—either that contained in the hog FSH preparation or the patients' endogeneous ICSH. Increased estrogen excretion and enlargement of the ovaries, as seen in our patients, have also been observed in women receiving pregnant mare serum gonadotropin (10), a hormone having actions similar to pituitary FSH. Cystic follicular enlargement of the ovaries has been observed in patients receiving sheep pituitary FSH, alone (11) or in combination with human chorionic gonadotropin (12).

Confirming other evidence of increased estrogen secretion, we found large amounts of estrogen in the ovarian-cyst fluid obtained from a patient receiving hog FSH. We are not aware of information concerning the estrogen content of normal human follicular fluid, but MacCorquodale, Thayer and Doisy (13) in their studies leading to the isolation of estradiol, determined the estrogen concentration of the follicular fluid from the ovaries of the sow. Expressed as estradiol, the estrogen content of our patient's follicular fluid was ten times greater than that of the fluid from sow ovaries.

Progesterone effects where observed in 3 patients while they were receiving FSH. Two of these patients (S.A. and R.W.) received FSH during the second half of the menstrual cycle when progesterone secretion would be expected. In Patient M.C., however, endometrial biopsies and pregnanediol assays indicated that progesterone was secreted for several weeks. In animals, estrogens can stimulate pituitary secretion of ICSH and presumably of luteotropin, thereby inducing luteal development and secretion of progesterone (14). This could well have been the mechanism in Patient

M.C., since increased estrogen levels preceded and accompanied the changes indicative of progesterone secretion. An alternate explanation, that luteal function was stimulated by the small amounts of ICSH and luteotropin present in the administered FSH, seems unlikely, since large amounts of ICSH and luteotropin are necessary to stimulate luteal function in women (15, 16).

The ovarian-stimulating properties of FSH might reasonably be expected to benefit conditions such as ovarian deficiency associated with low gonadotropin excretion. The problems of cystic enlargement of the ovaries and of antihormone formation, however, present serious obstacles to effective therapy. In early clinical studies, chorionic gonadotropin was administered with the pituitary FSH, in the hope that the luteinizing properties of CG would prevent excessive follicular development. Although luteinization was induced, cystic enlargement still occurred (12). More careful adjustment of FSH dosage might avoid excessive follicular development; but in our patient having hypogonadotropic hypogonadism the dose of FSH necessary to increase estrogen excretion from low to normal levels resulted in abnormal ovarian enlargement.

Leathem (17) suggested that purification of gonadotropic hormone preparations might reduce their tendency to stimulate antihormone formation. Gordon (18), however, found that purification did not alter the antihormone-evoking properties of sheep pituitary gonadotropin. Our experience was similar; the patient who received the more purified preparation formed antihormones as rapidly as the patients who received FSH containing five times more solids per unit of hormone. Further experience with the purified preparation has confirmed that it readily stimulates antihormone formation. These observations suggest that hormone purification will not provide a practical solution to the problem of antihormone formation. Jungck and Brown (19) approached the problem by using human FSH prepared from postmenopausal urine. They administered this material to a patient for four months without evoking antihormone formation. The inherent difficulties in preparation, however, limit this theoretically feasible approach.

The antihormones which formed in patients receiving hog FSH prevented in rats the actions of both human pituitary gonadotropin and hog FSH. This was reflected by inhibition of the patients' response to both administered and endogenous FSH. Inhibition of exogenous FSH was manifested by a disappearance of stimulatory effects during the second or third month of the initial course of treatment and by a complete lack of stimulatory effects with retreatment. Neutralization of the patients' endogenous FSH resulted in a fall in urinary estrogens to below pretreatment levels, amenorrhea and, in 1 case, menopausal symptoms. Suppres-

sion of pituitary FSH secretion by the previously elevated estrogen levels could account for the initial inhibition of ovarian function. These inhibitory changes persisted much longer, however, in the patients who formed antihormones than in those who did not. The inhibition of endogenous FSH was most clearly seen upon retreatment, at which time antihormones rapidly increased and estrogens promptly fell to low levels.

Although antihormone formation prevents effective long-term FSH therapy, in certain situations it may have useful applications. In an editorial entitled "The Need for an Inhibitor of Gonadotropin" Wilkins (20) stated " . . . it would be most desirable to find some method of suppressing the secretion of pituitary gonadotropins. This might lead to the prevention or control of sexual precocity of the "constitutional type," the correction of certain menstrual and menopausal disorders, the regulation of fertility and the suppression of some gonadal neoplasms." In the present study, gonadotropins were inhibited by antihormones. The stimulatory effects which occurred prior to the formation of antihormones are obviously undesirable in situations such as constitutional precocious puberty—a syndrome in which inhibition of ovarian function is the objective of therapy. In recent studies by Paulsen, stimulatory effects were avoided and antihormone formation was induced by administering FSH once weekly for two to three months (21).

The period of observation in these studies was too short to determine if permanent benefit was achieved by temporarily inhibiting ovarian function in the girl having constitutional precocious puberty and the patient having functional bleeding. The results suggest, however, that the antihormone-evoking properties of FSH may prove more useful than its ability to stimulate ovarian function.

SUMMARY AND CONCLUSIONS

The administration of pituitary follicle-stimulating hormone (FSH) to 9 female patients resulted in a prompt increase of urinary estrogen excretion in the 7 having functioning ovaries, but not in the 2 castrates. Increased urinary estrogen levels were associated with cystic follicular enlargement of the ovaries and changes in the breasts indicative of increased estrogen secretion.

Antihormones, which were capable of inhibiting human pituitary FSH as well as the administered hog FSH, were formed in each of the 4 patients who received treatment for periods of two months or longer. Two of these patients, a 7-year-old girl having constitutional precocious puberty and an 18-year-old girl having severe functional bleeding, were individuals in whom temporary cessation of ovarian function was considered desirable. Neutralization of exogenous FSH was manifested by a disappearance of

stimulatory ovarian effects during the second or third month of therapy and by a complete lack of such stimulatory effects with retreatment. Inhibition of the patients' endogenous FSH was reflected by a fall in urinary estrogens to below pretreatment levels, amenorrhea and, in 1 case, menopausal symptoms. The inhibition of endogenous FSH was seen most clearly upon retreatment, at which time antihormones rapidly increased and urinary estrogens fell to low levels.

It is concluded that the induction of antihormone formation by the administration of pituitary FSH may have therapeutic application in cases in which it is desirable to inhibit pituitary gonadotropin.

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SALIVARY AND THYROIDAL RADIOIODIDE CLEARANCES OF PLASMA IN VARIOUS STATES OF THYROID FUNCTION

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IT IS now well established that the parotid gland concentrates plasma iodide to an extent comparable with that of the thyroid gland (1–3). The hypothesis of the identity of the trapping mechanisms in the parotid and in the thyroid is supported by the observations that the iodide concentration gradients between the glands and the plasma are about the same (2–4) and that both gradients can be reduced to almost zero by the administration of thiocyanate, perchlorate, or nitrate (4–8).

Recently Thode *et al.* (9) reported that the ratio of salivary to plasma I^{131} concentrations measured in samples of plasma and of mixed saliva (serous+mucous) collected twenty-four hours following administration of a tracer dose, is a valuable index of thyroid function. When the salivary radioactivity was compared with the activity of the precipitable plasma proteins instead of with that of whole plasma, an even better discrimination between normal and pathologic states of thyroid function was obtained. From these results the authors concluded that "a definite relationship exists between thyroid and submaxillary gland function" and that "the submaxillary gland not only concentrates iodide ion but is concerned with thyroxine as well, perhaps in its deiodination."

We thought that the validity of this hypothesis could best be tested by comparing the thyroidal and salivary radioiodide clearance of plasma in a number of patients with varying degrees of thyroid dysfunction. During the course of this investigation some additional experiments concerning the behavior of the radioiodide clearance by the thyroid and the parotid gland during the early phase of iodide distribution were carried out, involving the use of a newly available radioactive isotope of iodine, I^{132}.

METHODS

The 23 subjects studied were hospitalized patients whose thyroid function has been previously established by clinical observation and various

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laboratory tests, including three different radioiodine tests: the 24-hour thyroidal uptake, the concentration of serum protein-bound I^{131} (PBI^{131}) in a 48-hour plasma sample, and the so-called "radioiodine plasma test" which, in our hands, was found to be a reliable index of thyroid function in most instances (10).

Radioiodide (I^{131} or I^{132}) containing a carrier of about 10 μg. of KI^{127} was given either orally or intravenously, depending upon the type of study, in a dose of 100 μc. An equal amount was put into a glass model imitating a human thyroid weighing about 25 Gm., the model being immersed in a beaker of water. This standard and the amount of radioiodide retained by the thyroid was measured with a collimated scintillation counter fitted with a $1\frac{1}{2}\times 1$ inch thallium-activated sodium iodide crystal at a skin-crystal (or glass-crystal, respectively) distance of 37 cm. A 1-mm. lead filter was used to correct for weak scatter radiations. A third dose was diluted with tap water in such fashion as to attain a final concentration of 0.1 per cent of the dose in 10 ml. This standard and the 10-ml. plasma and urine samples were measured in a flat-bottomed glass cup put on top of the crystal. With this arrangement, the counting rate for 1 μc. of I^{131} in 10 ml. was about 3×10^5 cpm.

Both isotopes of iodine were obtained from A.E.R.E., Harwell, England. I^{132} was milked daily from a 20-mc. Te^{132} source by a distillation method, the tellurium being present as an acid solution containing 10 per cent sulphuric acid, 10 per cent phosphoric acid and sufficient N/10 potassium permanganate to render the solution a permanent pink color. Following addition of a small carrier, N/10 hydroxylamine sulphate was added until decolorization occurred, and the iodine set free was distilled over into a small amount of N/100 Na_2SO_3. By serial measurements of physical decay, and after corrections for the small amount (less than 1 per cent) of I^{131} present were applied, a value of 138 min. for the physical half-life of I^{132} was obtained, which is half-way between the values given by American (11) and British (12) workers. This value was, therefore, used in the I^{132} studies, to correct for physical decay.

For calculation of thyroidal iodide clearance, two methods were employed. The direct method was that of Berson *et al.* (13), with the exception that the disappearance curve of radioiodide from the plasma was determined separately in each patient. Starting three minutes after intravenous injection of the tracer dose, several blood samples were drawn and frequent measurements of the neck radioactivity were made for the following two hours. The "thyroidal uptake" at three minutes was assumed to represent extrathyroidal radioiodide concentration in the blood and in neck tissues. After plotting the plasma radioiodide concentrations on semi-log paper, a smooth curve was drawn through all the points and the neck activities were corrected for extrathyroidal activity, which was assumed to decrease parallel to the plasma concentration. The values for "true" thyroidal uptake thus obtained were also plotted on semi-log paper and again a smooth curve was drawn through these points. The fraction of the dose taken up by the thyroid during the period under consideration was calculated by subtracting the uptake value at the beginning of the period from that at its end. Direct clearance, expressed as milliliters of plasma cleared per minute, was then calculated by dividing this fraction by the plasma radioiodide concentration at the mid-point of the period obtained from the smoothed plasma concentration curve. Oddie *et al.* (14) have pointed out that during the time of distribution of the administered radioiodide within the iodide space of the body, the extrathyroidal neck activity behaves somewhat differently from the plasma activity, due to diffusion of the radioiodide into the tissues covered by the counter; however, with

proper shielding, the count for extrathyroidal activity should be but a small fraction of the count for total neck activity, and therefore the errors involved should not be unduly large.

The other method of calculating thyroidal clearance was an indirect one, using the renal clearance and the disappearance rate of radioiodide from the plasma. The renal clearance was estimated according to Berkson *et al.* (15), with the simplification that instead of taking several urine specimens and of extrapolating to the final excretion value, the amount of radioiodide excreted during the first forty-eight hours, E, was assumed to represent that fraction of the administered dose which was not taken up by the thyroid. Then the fraction taken up by the thyroid, U, is

$$U = 1 - E.$$

Since under the present experimental conditions the fraction of the dose which was excreted into the parotid saliva during the period of 120–180 minutes following administration, S, did not return to the body, the uptake, U, was to be calculated from

$$U = 1 - (E + S).$$

During the period of saliva collection, radioiodide was removed from the iodide space along three different routes: by thyroidal uptake with a rate constant K_{IT}, by urinary excretion with a rate constant K_{IE}, and by salivary excretion with a rate constant K_{IS}. The constant of the disappearance rate from the iodide space is then the sum of the single rate constants:

$$K_I = K_{IE} + K_{IT} + K_{IS}.$$

By plotting the radioiodide plasma concentrations at the beginning and at the end of the collection period (i_2 at 120 minutes, and i_3 at 180 minutes) on semi-log paper and assuming that at 120 minutes distribution of the administered dose within the iodide space has already been completed, this rate constant, K_I, could be calculated from the rate of disappearance of the radioiodide from the plasma during this period. During the first two hours, that is before saliva collection was started, radioiodide disappeared theoretically from the plasma with a rate constant ($K_{IE}+K_{IT}$) only and this, by definition, could be calculated from

$$K_{IE} + K_{IT} = (1 - S) \cdot K_I.$$

The plasma radioiodide concentration at zero time, i_0, (that is, if distribution of the dose within the iodide space would have been instantaneous) is arrived at by extrapolating back to zero time from the value of i_2 with a line the slope of which corresponds to the rate constant ($K_{IE}+K_{IT}$). The volume of the iodide space, V_I, can then be calculated by dividing the administered dose, *i.e.*, unity, by this value, or

$$V_I = \frac{1}{i_0}.$$

According to Berkson *et al.* (15) the renal clearance, C_E, can be calculated from the disappearance rate constant, from the volume of distribution, and from the fraction of the dose excreted into the urine:

$$C_E = E \cdot V_I \cdot (K_{IE} + K_{IT}).$$

Similarly, the thyroidal clearance, C_T, can be calculated from

$$C_T = U \cdot V_I \cdot (K_{IE} + K_{IT}).$$

It is realized that the inaccuracy of these calculations might be appreciable, especially when iodine metabolism is rapid and part of the dose excreted into the 48-hour urine is

already derived from the breakdown of radioactive hormone, but it should not be large enough to invalidate the conclusions to be drawn.

Samples of pure serous saliva were obtained by inserting a small polyvinyl catheter into either the right or left parotid duct. A small test tube was attached to its distal end, thereby securing complete collection of the volume of saliva secreted by one of the parotid glands and avoiding contamination with mixed saliva from the mouth. The volume of the saliva secreted, at times rather small, was measured with a precision pipet and diluted up to 10 or even 100 ml., depending upon its radioiodide content. Salivary clearance was always calculated by the direct method, dividing the amount excreted during the collection period by the plasma concentration at the mid-point of this period.

When mixed saliva was to be collected for the 24-hour S/P-ratio, patients were asked to spit freely into a suitable tube. A blood sample was drawn when half the required amount of saliva was delivered.

Plasma radioiodide concentration was determined, after precipitation of the plasma proteins with trichloroacetic acid, by measuring the radioactivity of the supernatant liquid and of two washings of the precipitate. Protein-bound radioactivity was measured after redissolving the washed precipitate with caustic soda. Urine was collected in bed pans which contained a drop of strong KI solution. It was assumed that all urinary radioactivity was present in the form of iodide. At least 10,000 counts were made in all measurements.

RESULTS

Comparison of salivary and thyroidal clearances

Ten subjects were studied. In 2, thyroidal function could be considered to be normal; in 1, there was impaired renal function but thyroidal function was normal; 1 patient had clear-cut myxedema; 2 had moderate thyrotoxicosis; and 4 had nontoxic goiter, caused in 2 instances by chronic iodine deficiency (in 1 of these the experiment was repeated during propylthiouracil therapy).

TABLE 1. COMPARISON OF SALIVARY, RENAL, AND THYROIDAL RADIOIODIDE CLEARANCE OF PLASMA

	Diagnosis	S*	E	U	i_2	i_1	i_0	V_I (liters)	$K_{IS}+K_{IE}+K_{IT}$ (proportion/hr.)	C_S	C_E	C_T
		(% of dose)			(% of dose/liter)					(ml./min.)		
1	Myxedema	12.19	76.8	11.01	3.56	3.11	4.52	22.1	0.1385	61.0	34.4	4.9
2	Normal thyroid function	7.95	42.65	49.4	2.11	1.97	2.39	41.8	0.0675	65.0	18.5	21.4
3	Normal thyroid function	5.1	48.6	46.3	2.46	2.11	3.29	30.4	0.1512	37.1	35.5	33.8
4	Thyrotoxicosis	1.16	10.44	88.4	0.75	0.47	1.89	52.9	0.231	32.2	21.0	177.6
5	Thyrotoxicosis	1.82	18.9	79.28	1.13	0.60	3.91	25.6	0.630	36.3	49.7	209.2
6	Normal thyroid function; glomerulo-sclerosis	14.0	41.6	44.4	4.57	4.28	5.09	19.6	0.068	53.0	8.0	8.6
7	Nontoxic goiter	8.75	17.55	73.7	2.08	1.73	2.87	34.8	0.182	77.5	16.9	71.0
8	Nontoxic goiter	6.8	28.9	64.2	1.73	1.43	2.99	33.4	0.277	44.0	41.6	92.5
9	Chronic iodine-deficient goiter	1.95	8.6	89.85	0.40	0.30	0.62	161.1 (?)	0.216	75.0	50.1	524.5
10a	Chronic iodine-deficient goiter	4.85	28.4	66.75	2.26	1.58	4.51	22.1	0.366	42.7	36.5	85.9
10b	Chronic iodine-deficient goiter treated with propylthiouracil	7.79	59.5	32.71	3.16	2.85	3.71	27.0	0.0924	43.2	22.8	12.5

* For explanation of symbols see under Methods.

Collection of serous saliva was started at 120 minutes following oral administration of the tracer dose of radioiodide and continued for 60 minutes, blood samples being drawn at the beginning and at the end of the collection period (i_2 and i_3). Urine was collected for forty-eight hours.

The results are presented in Table 1. In Figure 1 the salivary clearances are plotted against the thyroidal clearances of the same patients and it will be seen that, within the limits of experimental error, the salivary clearance remained fairly constant over a wide range of thyroidal clearances.

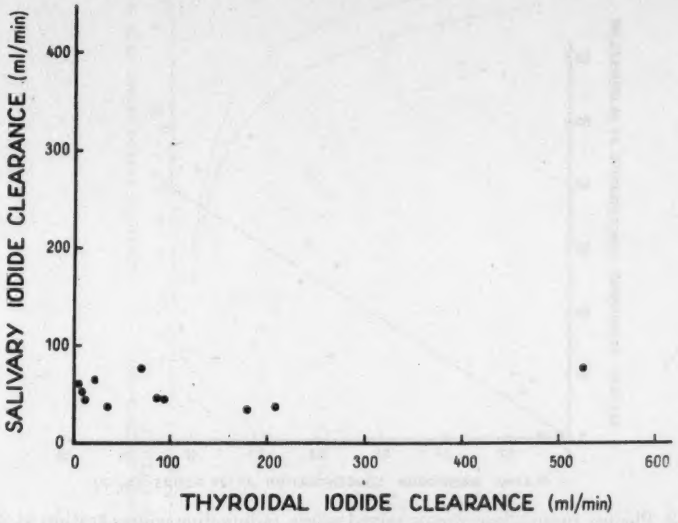

FIG. 1. Thyroid-gland versus parotid-gland radioiodide clearance of plasma.

The ratio between radioiodide concentrations in mixed saliva and plasma (S/P-ratio)

In the foregoing patients and in some additional subjects, mixed saliva was collected and a blood sample was drawn twenty-four hours after administration of the tracer dose. This time was chosen in order to permit direct comparison with the results of Thode *et al.* (9), and because of the suggestion of these authors that several hours after administration of the tracer dose would have to elapse before significant differences between the S/P-ratios in different states of thyroid function could be observed.

In Figure 2 the radioiodide concentrations of mixed saliva are plotted against the radioiodide plasma concentrations. The regression line, determined by the method of least squares, passes through the origin and corresponds to a concentration gradient of 30.8:1. It should be noted that there was a wide variation in salivary flows.

The behavior of the thyroidal clearance during the early phase of iodide distribution

At this stage of the investigation we had available some results of double experiments with I^{132} on the behavior of the thyroidal clearance during the first two hours following administration of the tracer dose. Thyroidal clearance was determined by the direct procedure, as described under Methods. The experiment was repeated twenty-four hours later under conditions as similar as possible (Fig. 3). Five periods of time were then chosen for calcu-

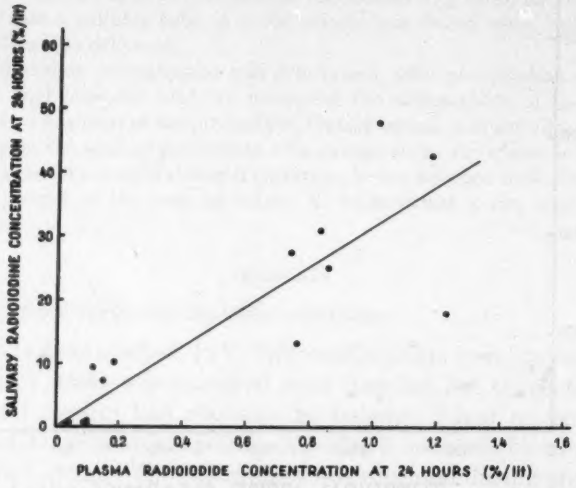

FIG. 2. Plasma radioiodide versus mixed saliva radioiodine concentration at 24 hours, following administration of a tracer dose. The scatter is mainly due to large variations in salivary flow.

lation of thyroidal clearance: 10–30; 31–50; 51–70; 71–90; and 91–120 minutes after injection.

The results of 3 such double experiments are presented in Table 2, in addition to the results of 2 experiments in which I^{131} was used only once. In all experiments it was observed that the thyroidal clearance did not remain constant but decreased significantly, usually for at least 90 minutes following administration of the dose.

The behavior of the salivary clearance during the early phase of iodide distribution

In these experiments I^{131} was used. Parotid saliva was collected for periods which corresponded to those chosen in the foregoing experiments. Saliva collected during the first 10 minutes following injection was dis-

carded. Salivary clearances were calculated in the same manner as the thyroidal clearances. The results of 4 such experiments are presented in Table 3. In Figure 4 the thyroidal as well as the salivary clearance values, calculated from 31 minutes following administration onwards, have been expressed as percentages of the clearance value during the 10–30 minute

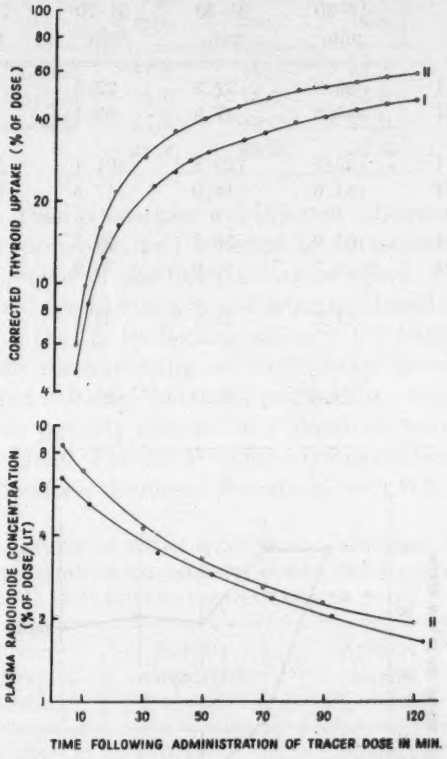

FIG. 3. Serial measurements of thyroidal uptake and plasma concentration of I^{132} on two successive days in a thyrotoxic subject (Case 2 of Table 2). The thyroidal uptake data are corrected for extrathyroidal neck radioactivity.

period. It will be seen that, in contrast to the thyroidal clearance, the salivary clearance decreased only slightly on the average, and with doubtful significance.

The influence of salivary flow on the S/P-ratio

Table 4 lists the results of 2 experiments which were performed forty-eight hours after administration of a tracer dose of I^{131}. Parotid saliva was collected for 10 minutes and a blood sample was drawn at the mid-point

TABLE 2. THYROIDAL RADIOIODIDE CLEARANCE OF PLASMA DURING EARLY PHASE OF IODIDE DISTRIBUTION

In the first 3 subjects the experiment (I) was repeated after twenty-four hours (II) under conditions as similar as possible, using I^{132}. In the remaining 2 cases, I^{131} was used.

Diagnosis		Volume of plasma (ml.) cleared per minute in period of time (min.) after administration				
		10–30 min.	31–50 min.	51–70 min.	71–90 min.	91–130 min.
1 Normal thyroid function	I	50.0	32.3	23.5	25.1	26.4
	II	45.6	37.9	30.4	24.4	25.8
2 Thyrotoxicosis	I	153.9	129.3	101.1	112.7	97.2
	II	184.0	144.0	117.8	100.0	85.9
3 Nontoxic goiter	I	105.0	78.2	74.5	65.0	64.5
	II	115.7	95.0	78.9	67.9	81.5
4 Normal thyroid function		41.2	24.2	27.8	28.7	27.4
5 Normal thyroid function		42.5	25.6	22.6	18.2	15.4

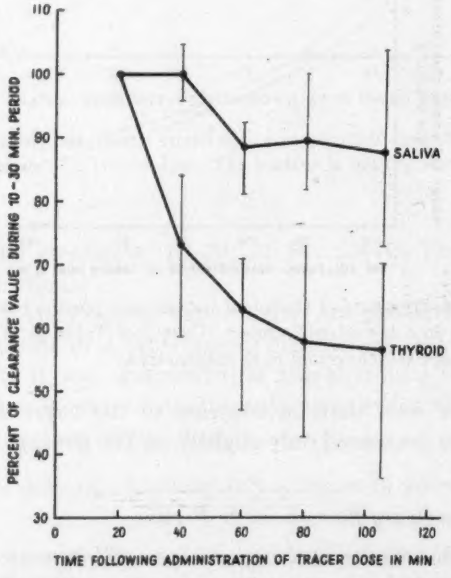

FIG. 4. All thyroidal and salivary clearance values obtained during the 10–30 minute period have been taken as 100, and the values obtained during the following periods have been expressed as a percentage thereof.

TABLE 3. SALIVARY RADIOIODIDE CLEARANCE OF PLASMA DURING EARLY PHASE OF IODIDE DISTRIBUTION

Diagnosis	Volume of plasma (ml.) cleared per minute in period of time (min.) after administration				
	10–30 min.	31–50 min.	51–70 min.	71–90 min.	91–120 min.
1 Normal thyroid function	51.6	49.5	47.8	51.8	53.8
2 Normal thyroid function	15.0	15.7	14.0	14.3	13.3
3 Normal thyroid function	45.5	44.2	37.0	38.3	33.8
4 Juvenile goiter	24.4	26.2	22.4	21.0	21.2

of this period (I). Then pilocarpine was injected subcutaneously in doses of 8 and 12 mg., respectively, and a period of 10 minutes was allowed to elapse for development of the full pilocarpine effect. Parotid saliva was then again collected for 10 minutes and a further blood sample was taken (II). It was found that in both cases salivary I^{131} concentration did not decrease at a rate corresponding to the increase in salivary flow. The amount of I^{131} excreted was, therefore, greater than that excreted during the control period, possibly because of a simultaneous increase in blood flow through the gland. Plasma I^{131} concentrations remained unchanged. The S/P-ratio, therefore, decreased sharply in both subjects.

TABLE 4. EFFECT OF PILOCARPINE ON SALIVARY FLOW, SALIVARY EXCRETION OF RADIOIODINE, AND ON THE S/P-RATIO AT 48 HOURS AFTER THE TRACER DOSE

Expt.		Salivary flow ml./min.	Salivary concentration (% of dose/L)	Amount excreted (% of dose)	S/P-Ratio
1	I	0.078	0.44	0.000343	1.37
	II	0.63	0.124	0.000781	0.395
2	I	0.268	0.615	0.00165	2.31
	II	3.26	0.0765	0.00249	0.288

DISCUSSION

The results of the present experiments do not support the view that thyroidal and submaxillary gland functions are related to each other. As far as the concentration mechanism of the salivary glands for plasma iodide is concerned, no indication was obtained that it is influenced by thyroidal function. The present results do not exclude the possibility that the sali-

vary glands play an important rôle in the degradation of thyroxine; however, the assumption that the S/P-ratio is determined by such a process had been rendered highly improbable by theoretical considerations recently presented by Freinkel and Ingbar (16).

If it be true that the salivary iodide clearance is independent of thyroidal function and if it be, furthermore, assumed that also salivary flow is independent of thyroidal function, then the concentration of salivary radioiodide should be proportional to the plasma radioiodide concentration. From Figure 2 this appears to be the case, if due allowance be made for the large variations in salivary flow, which in our experiments ranged from 0.15 to 17.1 ml. of serous saliva per hour. The average concentration gradient of about 31 is in close agreement with that previously observed by other groups (2, 17, 18). By calculating from the data of Thode *et al.* (9) the saliva-plasma ratios of radioiodide, Pochin (19) and Freinkel and Ingbar (16) have demonstrated that a similar concentration gradient for iodide accounts for the S/P-ratios of radioiodine observed by Thode *et al.* in normal and hyperthyroid cases.

Thus it can be concluded that the S/P-ratio of Thode *et al.* is mainly determined by the behavior of the plasma radioiodide concentration. The observation that the S/P-ratio determined about 30 minutes after administration of the tracer dose does not correlate with thyroidal function (3) might be explained by the fact that after so short a time the plasma radioiodide concentration deviates significantly from its normal value only in cases of extreme thyroidal hypo- or hyperactivity. Though some increase in the accuracy of the S/P-ratio in discriminating between hypo- and hyperfunction of the thyroid might be gained by using the plasma concentration of protein-bound radioiodine instead of the radioiodine concentration in whole plasma, it should be realized that the S/P-ratio must be influenced by the same factors as the plasma concentrations of radioiodide and of protein-bound radioiodine. As Riggs (20) has shown, these factors are (besides the hormonal secretion rate) the daily intake of iodine, its fecal excretion, the renal iodide clearance, the volumes of the iodide and of the hormonal iodine spaces, and the iodine content of the gland. Thus, for instance, falsely high values would be obtained in cases with impaired renal function in which plasma radioiodide concentration is high, and falsely low values in cases of chronic iodine deficiency in which plasma radioiodide concentration is low. In both instances the hormonal secretion rate is, or can be normal. Similarly, falsely low values would be obtained in thyroidectomized or radioiodine-treated patients in whom the PBI^{131} level is, or can be high without any increase in hormonal secretion above normal (21).

In addition, the value of the S/P-ratio depends upon two further extrathyroidal factors, *i.e.*, upon the extent of dilution of serous by mucous

saliva and upon the rate of salivary flow. Honour *et al.* (2) have noted that, on the average, pure serous saliva contains 2.7 times as much radioiodide as does mixed saliva, probably because the iodide-concentrating mechanism is confined to the serous variety of the saliva-secreting cells. It is obvious that any variation in the proportions of serous and mucous saliva must affect the S/P-ratio. Honour *et al.* have measured the salivary radioiodide clearance in mixed saliva and have found an average value of 17.5 ml. per minute. If this value is multiplied by the factor 2.7, one arrives at a value of 47.3 ml. per minute for clearance in serous saliva, which is very close to the average value of 46 ml. per minute at two hours, observed in the present experiments. The dependence of the S/P-ratio upon the rate of salivary flow has been demonstrated in Table 4.

The regular finding of a decreasing thyroidal clearance is in contrast to the data of Berson and Yalow (22), who reported the thyroidal clearance to remain constant. Nodine *et al.* (23) have observed that in normal young persons the thyroidal clearance during the period of 24–72 hours after administration of the tracer dose is, on the average, lower than during the period of 2–12 hours. The present experiments extend these observations to the period of 10–120 minutes, and confirm that the thyroidal clearance decreases even more sharply during the early phase of iodide distribution. These results could be explained by the hypothesis that, in cases with normal production of hormone, the rate of iodine binding in the thyroid is slower than the rate of iodide trapping by the gland. The volume of the iodide space within the thyroid would determine at which time after administration of the dose the rate of trapping is forced to slow down to the rate of binding. If this be true, then the administration of thiocyanate or of a similarly-acting substance within, say, thirty minutes after the tracer dose, should cause a decrease of thyroidal radioactivity due to the discharge of the trapped but still unbound radioiodide from the thyroidal iodide space. In fact, such an effect has been frequently seen in this laboratory following a dose of perchlorate. It should be noted, however, that the present experiments were performed in a region of chronic iodine deficiency where diffuse and adenomatous goiters are so common that it is sometimes difficult to find for experimental purposes a subject, the size and function of whose thyroid could with safety be regarded as truly normal. The present results should, therefore, be treated with reserve and should await confirmation by similar experiments performed in a region of iodine abundance.

Berson and Yalow (22) made their observations of a constant thyroidal clearance in thyrotoxic subjects in whom it can be assumed that, because of the increase in formation of hormone, the rate of binding should either exceed the rate of trapping or should (since the rate of trapping is probably also enhanced in this condition) at least not be slower. It would then al-

ready be the rate of binding which is measured during the first thirty minutes. The apparent constancy of the thyroidal clearance could further be explained by the observation made by Ingbar (24) of a larger thyroidal iodide space in this condition. In that case it might take longer than the period during which the estimations of the thyroidal clearance were made before the thyroidal iodide space is completely filled up and the rate of trapping is reduced to that of binding. If this explanation should be valid, then a dose of thiocyanate should cause a significant discharge of radioiodide from the thyroid; with one report denying (22) and the other confirming (24) such an effect, it is difficult to answer this question.[1]

If it be true that the trapping mechanisms in the parotid and in the thyroid gland are identical, the hypothesis that in normal cases the rate of trapping exceeds the rate of binding is supported by the observation of an average salivary clearance value of 46 ml. per minute, which is about three times the average normal value for thyroidal clearance of 17 ml. per minute. The latter value was usually obtained under conditions where equilibrium with the thyroidal iodide space was already attained and the thyroidal clearance was, therefore, determined by the rate of binding.

In contrast to the thyroidal clearance, the salivary clearance remains either constant or decreases only slightly and with doubtful significance. Apparently, the rate of iodide excretion into the saliva is very close to the rate of trapping. Rowlands *et al.* (7) have observed that the "dead-time" between the presence of radioiodine in the plasma and its appearance in saliva in the collecting tube is less than one minute. This has been confirmed in some of our own experiments. In view of the delay caused by the sampling process through the catheter, it can be assumed that the transfer of iodide from plasma to saliva is instantaneous and that the volume of the iodide space within the parotid gland is negligible.

The double experiments with I^{132} confirmed the report of Hanbury *et al.* (25) that the results of the early uptake tests may vary considerably. The maximum variation in thyroidal clearance in the same patients was found to amount to 26 per cent of the mean. Incidentally, this value is identical with the maximum variation in the 1-hour thyroidal uptake observed by Hanbury *et al.* (25). It is obvious that the possibility of a variation of this magnitude should be kept in mind if the results of early radioiodine tests are used in the diagnosis of thyroid disorders.

SUMMARY

1. In 10 patients with various states of thyroid function the radioiodide clearance of plasma by the parotid gland was observed to be independent of thyroidal function and to average 46 ml. per minute.

[1] In the thyrotoxic subject of Table 2 the thyroidal clearance was certainly falling.

2. The ratio of salivary to plasma radioiodine concentration is apparently determined by the plasma concentrations of radioiodide and protein-bound radioiodine, and reflects thyroidal function only in so far as the plasma concentrations do. In addition to all the factors which determine the plasma concentrations, the saliva/plasma ratio is influenced by the rate of salivary flow and by the extent of dilution of serous by mucous saliva.

3. In contrast to the salivary radioiodide clearance, the thyroidal radioiodide clearance was found to decrease significantly during the early phase of iodide distribution. This observation can be explained by the hypothesis that thyroidal uptake is a two-rate function with, in normal cases, the rate of iodine binding being slower than the rate of iodide trapping.

4. Double estimations of the thyroidal clearance with I^{132} have confirmed previous reports that iodine metabolism in its early phase may be subject to considerable daily variations.

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SERUM INORGANIC PHOSPHORUS LEVELS IN MALES AND FEMALES AT PROGRESSIVE AGES, WITH CONCOMITANT MEASUREMENTS OF URINARY KETOSTEROIDS AND ANDROGENS IN MEN*

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THE level of serum inorganic phosphorus (SIP) has been observed to increase after castration of young adult males (1). As tested in 9 subjects, the mean change reached statistically significant levels within fifteen days following orchiectomy and persisted for four years, at which time the study was discontinued. Six of the 9 subjects exhibited marked increases in SIP; the remaining 3 along with 3 males in another series, had high values prior to castration, and removal of their testes resulted either in only slight elevation of the SIP level or a decrease after three or four years.

These observations focussed attention upon the relationship of the SIP level to gonadal secretions in intact subjects and it was indicated that removal of gonadal secretions appeared to influence SIP concentration to a greater extent in young adults than in old persons (1). Since there seem to be no reports that compare SIP levels in young, middle-aged and old adults, or analyze the relationship between values for SIP and gonadal secretions, a study was made (a) of SIP levels at progressive ages in males and females, along with (b) simultaneous measurements of urinary ketosteroids[1] and androgens in some of these subjects.

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[1] The term "ketosteroids" is employed instead of the more commonly used designation of "17-ketosteroids" because the compounds measured in these assays include 3-, 17-, and 20-ketosteroids (2).

MATERIALS AND METHODS

Subjects consisted of 188 Caucasians—125 males who were 16 to 85 years of age, and 63 females who were 13 to 67 years old. All persons were apparently in good health. Blood was obtained when the subjects were in a basal state and SIP was measured according to the procedure described by Fiske and SubbaRow (3), adapted for use with a photoelectric colorimeter.

Urinary ketosteroids and androgens were measured in 45 of the men who were 21 to 75 years of age. Described elsewhere (4, 5) are the precautions observed in selecting this group of men, the methods employed, and the detailed results with statistical analyses. In brief, the urine was collected for ninety-six consecutive hours, extracted according to procedures described by Dobriner *et al.* (6) and assayed colorimetrically for ketosteroids (7). The extracts were assayed biologically for androgens, using the chick's comb (8) and Bliss' (9) statistical procedures. The curves of decline (4, 5) in values with progressive age are similar to those observed in other groups of aging men (10–15).

Studies were also undertaken to ascertain the reproducibility of the values obtained for SIP, ketosteroids and androgens.

RESULTS

Reliability of results. The range of values for different samples of blood from the same subjects is shown in Table 1 for 8 males and 7 females. The mean variation for samples taken from time to time was similar in males and females, σ being 0.47 and 0.43 mg. per 100 ml., respectively.

Seasonal influences were immaterial; this is in agreement with previous studies in which seasonal changes were found in small children (16) but not in either intact (16) or castrated (1) adults.

The validity of constructing curves throughout the lifespan (Figs. 1 and 2) by adding to our data those of Bullock (17) was tested by comparing the values over the range of years in which these data overlap. This was done for 99 males between 17 and 51 years of age and for 39 females 13 to 52 years old. The mean values for SIP concentration in these subjects, matched with regard to age, was 3.7 in both series of males, and 3.7 and 3.8 in females. The variation from subject to subject was similar for the two series of males; in females it was significantly greater in Bullock's series than in the present study.

The degree of variation in values for ketosteroids in repeated collections of urine from the same subjects has been described (4, 5). It was small in measurements of ketosteroids (± 0.8 mg. per twenty-four hours) but large in measurements of androgens (mean difference, 12 I.U. per twenty-four hours).

Influence of age. Values for SIP are high at birth and may become con-

TABLE 1. REPEATED MEASUREMENTS OF SIP (MG./100 ML.) IN THE SAME SUBJECTS

Males

A.S. (15–19 yrs.)	R.S. (20–24 yrs.)	D.E. (21–25 yrs.)	V.W. (27–29 yrs.)	B.A. (27–31 yrs.)	H.J. (32–34 yrs.)	R.H. (33–35 yrs.)	W.A. (62–64 yrs.)
—* 4.26	—* 3.00	—* 3.91	—* 3.95	—* 3.89	—* 3.76	—* 4.95	—* 3.24
460* 4.72	2* 3.29	93* 3.84	572* 3.50	84* 3.20	5* 4.12	630* 3.50	4* 3.50
111* 5.25	186* 3.17	48* 3.77		93* 3.34	7* 3.65	184* 3.40	7* 3.24
483* 4.45	4* 2.85	133* 2.91		147* 3.24	256* 3.50	21* 4.55	158* 2.56
259* 5.20	8* 3.52	91* 4.50		169* 3.37	867* 2.85	8* 3.35	111* 3.65
363* 4.32	113* 3.61	31* 4.50		91* 3.50			484* 3.80
	4* 3.11	893* 3.70		104* 2.92			471* 2.85
	9* 3.07	65* 3.85		246* 3.70			
	13* 3.56			1* 2.75			
	25* 3.61			235* 3.00			
	30* 2.60			219* 2.05			
	23* 4.22			202* 3.10			
	400* 3.35			97* 3.20			
	125* 3.80						
No. of samples 6	14	8	2	13	5	6	7
Mean 4.700	3.340	3.872	3.725	3.174	3.576	3.950	3.263
σ 0.4367	0.4182	0.5610	0.3183	0.4574	0.4659	0.7458	0.4397

Females

M.C. (20–23 yrs.)	J.L. (23 yrs.)	M.B. (24–28 yrs.)	G.C. (36–40 yrs.)	E.B. (38–41 yrs.)	C.A. (53–57 yrs.)	A.G. (54 yrs.)
—* 3.26	—* 3.57	—* 4.00	—* 3.80	—* 3.40	—* 3.90	—* 4.25
155* 2.91	89* 4.10	35* 4.60	236* 3.25	216* 2.46	595* 3.42	5* 3.51
26* 3.65		267* 4.28	3* 3.58	29* 3.30	111* 4.70	7* 4.30
52* 4.00		57* 3.84	102* 3.14	82* 2.12	484* 4.00	
188* 3.80		95* 4.40	34* 3.61	156* 3.74	471* 3.95	
188* 3.80		49* 4.13	35* 3.97	28* 3.40		
267* 4.20		609* 3.90	46* 2.90	70* 3.75		
		199* 4.70	43* 3.02	344* 3.05		
		271* 4.05	109* 3.80	364* 3.68		
			898* 3.90			
No. of samples 7	2	9	10	9	5	3
Mean 3.660	3.835	4.211	3.497	3.211	3.994	4.026
σ 0.4414	0.3748	0.3055	0.3890	0.5759	0.4579	0.4424

* Number of days between successive samples.

siderably higher if the baby is fed cow's milk, which contains much more phosphorus (P) than human milk (18).

Figures 1 and 2 show a decrease in the SIP level by the end of the first year but little further decline during the first decade of life. This is in agreement with the observations of Stearns and Warweg (19), in which mean values decreased only from 5.5 to 5.0 mg. per 100 ml. between the first and the twelfth years.

At sexual maturation or shortly thereafter, SIP is reduced to the range characteristic of adults (17, 19, 20). This reduction to adult levels has been

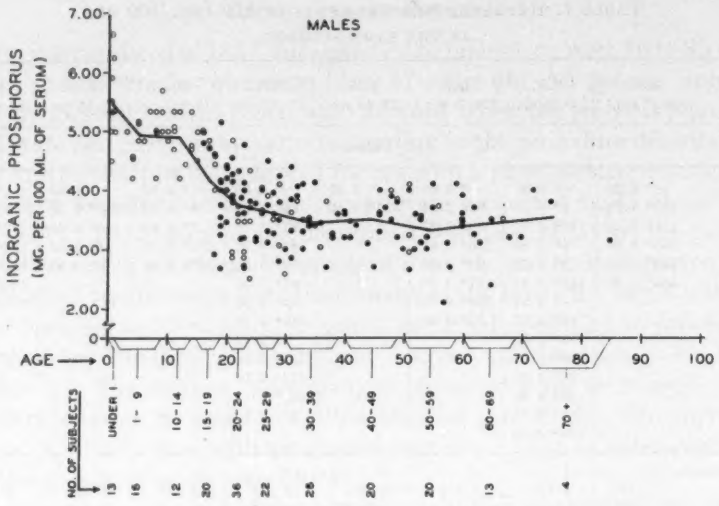

FIG. 1

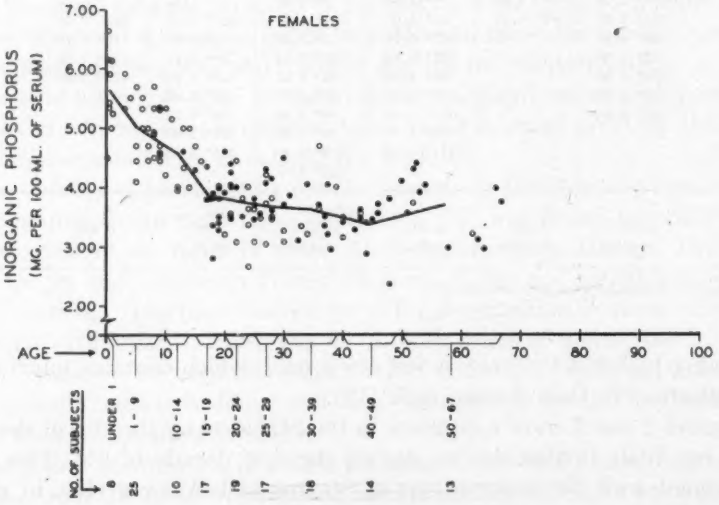

FIG. 2

FIGS. 1 and 2. Values for serum inorganic phosphorus (SIP) in males and females, respectively. Black symbols represent our data; white symbols, those of Bullock (17). Lines represent average values.

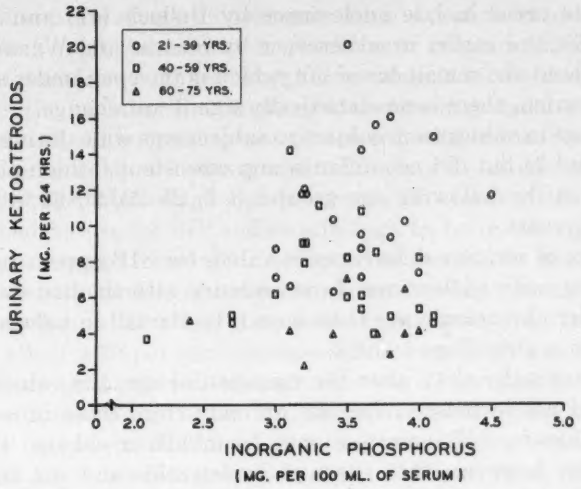

FIG. 3

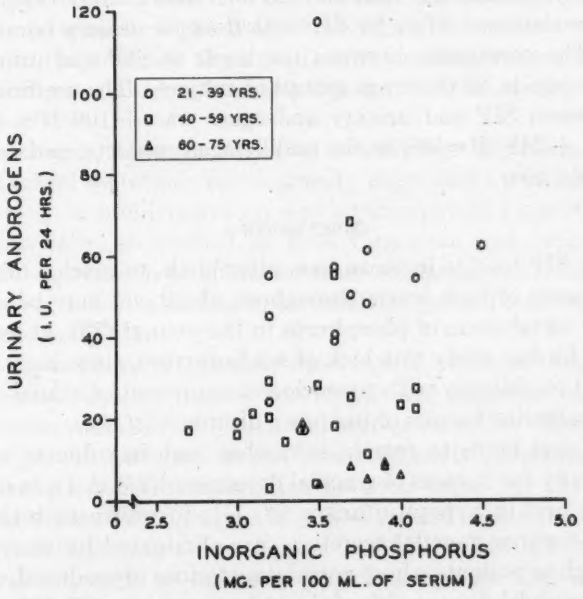

FIG. 4

FIGS. 3 and 4. Values for SIP were not correlated with titers of urinary steroids in normal men. The mean daily excretion was 12 mg. of ketosteroids (Fig. 3) and 47 I.U. of androgens (Fig. 4) in men 21 to 39 years old; 7 mg. and 23 I.U. in men 40 to 50 years old; and 4 mg. and 8 I.U. in men 60 to 75 years old.

reported to occur in late adolescence by Bullock (17) and Tisdall and Harris (20), and earlier in adolescence by Stearns and Warweg (19).

Throughout the remainder of life, which is the span under study in this communication, there is no statistically significant change.

The range in values from subject to subject was wide during adolescence (Figs. 1 and 2) but did not differ in any consistent fashion at other ages, as tested in the following age groups: 3–7, 25–29, 30–39, 40–49, 50–59, and 60–75 years.

Influence of sex. Sex differences in values for SIP appear to be notable only during early adolescence. In accordance with the maturation of girls at an earlier chronologic age than boys (21), the fall in values for SIP occurs earlier in girls (Figs. 1 and 2).

It is noteworthy that, after the menopausal age, the values for SIP in women did not increase or become different from those in men. Neither did the values for SIP increase in men by middle or old age. There can be little doubt, however, that titers of ketosteroids and sex hormones decrease by middle and old age. Tests demonstrated that drastic reductions had occurred in the middle-aged and old men under study (Figs. 3 and 4).

Lack of correlation of values for SIP with those for urinary ketosteroids and androgens. The correlation between the levels of SIP and urinary ketosteroids was zero in all three age groups of subjects. The coefficient of correlation between SIP and urinary androgens was +.109 (P = .65) in the young men, +.248 (P = .40) in the middle-aged subjects, and −.283 (P = .57) in the old men.

DISCUSSION

Values for SIP tend to increase soon after birth, to reach a lifetime high (17). Persistence of high levels throughout childhood may be due to the accentuated metabolism of phosphorus in the young[2] (22); at least it does not seem to be due solely to a lack of sex hormones, since high SIP levels are observed in children with precocious maturation of sexual characters because of endocrine tumors or unknown disorders (*cf.* 1).

The SIP level tends to remain somewhat high in subjects who fail to mature sexually (as in cases of gonadal dysgenesis (23; *cf.* 1), in eunuchoidism (24, 25) and in hypopituitarism (*cf.* 1)); to return to high levels in young adults whose gonadal secretions are eliminated by castration (1); and to be high in patients whose gonadal secretions are reduced, as in some cases of Simmonds' disease (26; *cf.* 1) and acromegaly (27, 28).

With advancing age, the low range of SIP values which was established

[2] "With increasing age the specific activity of organ phosphorus decreases. This is due partly to an increased phosphorus content and partly to a decreased turnover of phosphorus in the aging organism" (Hevesy (22) p. 109).

after sexual maturation seems to stem more from a decline in phosphorus metabolism (and/or an inherently increased rate of excretion of phosphorus) than it does from a depressive influence of gonadal secretions. This is obvious from the fact that the pronounced decline in gonadal secretions in middle-aged and old subjects is not associated with values for SIP as high as those found in all of 9 young adult eunuchs (1). In older eunuchs, 33 to 65 years of age, McCullagh and Renshaw (29) observed that only 4 of 9 subjects had values for SIP sufficiently high to be in the upper portion of the normal range for adults. Moreover, as studied in middle-aged and old subjects with cancer, who were considered to have no metastases and no deterioration in their condition while under study, castration resulted in only about a 20 per cent increase in SIP concentration (30). For comparison the mean increase after castration was 37 per cent in 9 young adult noncancerous subjects (49 per cent in the 6 of these young men who had low values before orchiectomy). As tested in cancerous eunuchs, old persons showed only moderate reduction of the SIP level upon treatment with sex hormones (30). That the reduction in SIP level was smaller when sex hormones were administered to old persons than when given to young subjects matured sexually is presumed to be related to the lower values for SIP apart from the influence of gonadal secretions and, consequently, less opportunity for gonadal secretions to effect further reduction in the concentration of SIP.

At these later ages, when the SIP level is neither greatly elevated in the absence of gonadal secretions nor markedly depressed by administration of sex hormones, the proliferative rates of integumentary appendages have decreased materially, as studied in both Caucasian and Japanese subjects (31). The decrease in proliferative rates of integumental appendages with age seems to reflect a decline in metabolism, growth and replacement of tissues in the body (31).

As discussed elsewhere (1), there is considerable evidence that the high SIP levels observed in some cases of acromegaly and gigantism may be due in part to concomitant failure of gonadal secretions rather than solely to high titers of growth hormone, as has been commonly supposed (28, 32).

The absence of significant correlation between values for SIP and for urinary ketosteroids and androgens is to be expected, even in young men in whom sex hormones may act to depress the SIP level. Studies of secondary sex characters, such as baldness (33), axillary hair (34) and beard (35), have shown that, although these characters are strictly dependent upon gonadal secretions for their development and maintenance, the *extent* to which they flourish is controlled by hereditary factors (36) and is not correlated significantly with values for urinary ketosteroids or androgens.

SUMMARY

Serum inorganic phosphorus (SIP) levels at progressive ages are described for each sex and analyzed with regard to variations from time to time in the same subjects and the range of values among subjects at progressive ages.

Previous studies have shown that the SIP level tends to be high until the age of sexual maturation or shortly thereafter, and that in young adults an inverse relationship exists between gonadal secretions and values for SIP. The present data indicate that the extent of the influence of gonadal secretions upon SIP concentration decreases with age, apparently due in part to low values for SIP apart from the influence of gonadal secretions and, consequently, less opportunity for gonadal secretions to effect further reduction.

Values for SIP are not correlated with urinary titers of ketosteroids and androgens, even in young men. This is in keeping with studies of secondary sex characters; the growth and maintenance of these characters are dependent upon stimulation by sex hormones, but the degree of their development is largely controlled by genetic factors and is not correlated significantly with values for urinary ketosteroids or androgens.

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STEROID STUDIES IN NORMAL AND ADIPOSE CHILDREN

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INTRODUCTION

SIMPSON (1) has described adiposity in children associated with other interesting clinical features, possibly referable to adrenocortical overactivity. Total neutral 17-ketosteroid estimations have been carried out on a small number of these children (2). However, the complex mixture of compounds estimated as 17-ketosteroids indicates the need for separation into the individual steroids, or at least into groups of steroids of related structure. Simpson (3) has also described an increased excretion of reducing steroids in some of these children whose position is on the border-land of endocrinopathy, *i.e.*, they demonstrate, in most cases, either a minimal pathologic condition or an extreme of physiologic normality.

Few studies of individual adrenocortical steroid excretion in children have been carried out using modern methods of investigation. The older methods of estimating total reducing steroids or total formaldehydogenic steroids are not reliable, since the estimations include nonsteroidal material. In the present studies, further investigation of the excretion of some individual adrenocortical steroids and 17-ketosteroids has been carried out in a small group of normal children and compared with similar studies in a group of adipose children in whom the diagnosis was adipose gynism and gynandrism (1). The excretion of individual 17-ketosteroid fractions as obtained by fractionation on alumina of the total urinary extract has now been studied in these children.

EXPERIMENTS AND RESULTS

The patients, who were selected from an out-patient clinic, resembled those originally described by Simpson (4). As far as possible, both investigations were carried out on the same patients, but there are several instances in which only one or other investigation was practicable.

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TABLE 1. THE EXCRETION OF ADRENOCORTICAL STEROIDS IN NORMAL AND ADIPOSE GIRLS (μG./24 HRS.)

Case No.	Age (yrs.)	Comp. X_4	Comp. F	Cortisone	Cortisone +Comp. F	Tetrahydro-cortisone
			Adipose gynism			
17	15	40	0	0	0	800
13	15	240	680	480	1,160	800
11	15	120	100	70	170	3,200
18	13	160	160	80	240	1,200
19	13	60	60	420	480	4,400
12	12	240	60	40	100	2,000
20	12	120	100	70	170	800
21	12	100	60	60	120	1,600
22	12	160	320	160	480	1,600
23	12	40	20	100	120	3,200
24	11	60	60	80	140	1,200
25	9	120	80	5	85	8,800
26	9	80	60	40	100	160
27	8	480	480	0	480	800
28	8	120	80	40	120	800
	Mean	*143*	*155*	*110*	*264*	*2,091*
	S.E.	*±28.9*	*±24.2*	*±37.4*	*±75.5*	*±564.9*
	Range	*40–480*	*0–680*	*0–480*	*0–1160*	*160–8,800*
			Normal girls			
42	7	32	20	32	52	40
43	7	20	10	20	30	40
44	9	40	10	20	30	200
45	11	80	60	20	80	1,200
46	11	60	10	40	50	200
47	13	80	40	80	120	1,600
48	14	40	40	60	100	240
49*	15	160	160	32	192	960
	Mean	*64*	*44*	*38*	*82*	*560*
	S.E.	*±15.7*	*±17.8*	*±7.7*	*±19.5*	*±213.6*
	Range	*20–160*	*10–160*	*20–80*	*30–192*	*40–1,600*

X_4	$t=1.889$;	$p=0.1-0.05$
F	$t=3.084$;	$p=0.01-0.001$
Cortisone	$t=1.377$;	$p=0.2-0.1$
Cortisone+Comp. F	$t=1.735$;	$p=0.1-0.05$
Tetrahydrocortisone	$t=1.921$;	$p=0.1-0.05$

* Manifestations of puberty present.

Adrenocortical steroids were measured by a modification of the method of de Courcy *et al.* (5). After extraction of the free steroid fraction with chloroform at pH 7, the urine was incubated with ox spleen β-glucuronidase (40,000 units per 24-hour specimen) to liberate steroids originally present as glucuronides. The incubated urine was then extracted with chloroform to yield the conjugated fraction. Free and conjugated fractions were run separately on paper chromatograms with benzene/50% methanol, known amounts of reference compounds being run on the same paper. After drying, the papers were treated with methanolic soda and triphenyltetrazolium

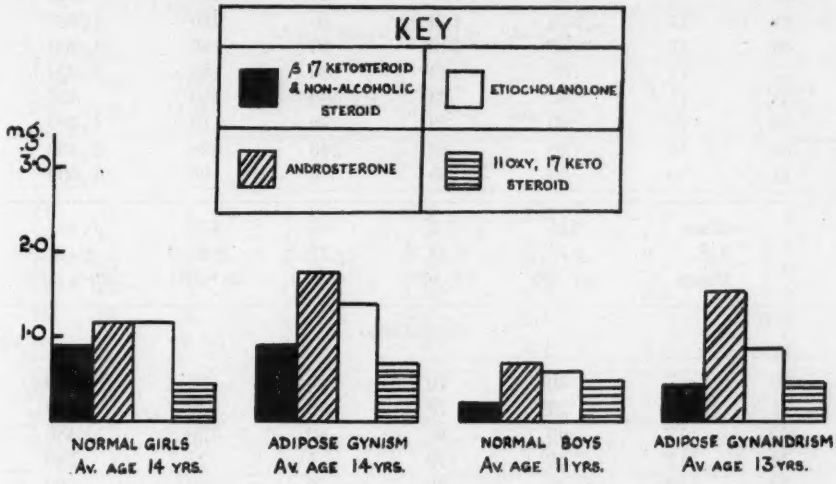

FIG. 1. Urinary steroids of 4 normal girls *vs.* 6 girls with adipose gynism; and of 6 normal boys *vs.* 5 boys with adipose gynandrism.

chloride. The size and intensity of the pink areas formed by the reducing steroids on the wet paper were compared with those formed by the known amounts of reference compounds. After drying, the size and intensity of the yellow fluorescent spots formed by the Δ^4-3-ketosteroids were also compared with those formed by the reference compounds. In this way it was possible to make semiquantitative estimations with a probable accuracy of about ± 20 per cent. The methods of identification of the spots have been discussed fully by de Courcy *et al.* (5). These identifications, which were not carried out on the individual cases, depended upon comparisons with reference compounds and on chromatographic behavior, with different solvents, of the free substances, their acetates and their oxidation products.

Cortisone, compound F, and a (so far, unidentified) Δ^4-3-ketosteroid,

TABLE 2. THE EXCRETION OF ADRENOCORTICAL STEROIDS IN NORMAL AND ADIPOSE BOYS (μG./24 HRS.)

Case No.	Age (yrs.)	Comp. X_4	Comp. F	Cortisone	Cortione +Comp. F	Tetrahydro-cortisone
			Adipose gynandrism			
29	20	120	20	200	220	2,400
30	15	40	10	140	150	4,800
34	13	120	80	40	120	320
35	13	160	160	40	200	800
31	12	220	140	80	220	1,200
36	11	120	100	80	180	4,000
37	11	160	120	60	180	1,200
38	11	60	20	80	100	800
39	10	60	20	90	110	1,200
40	10	120	80	240	320	2,400
41	9	90	80	10	90	1,600
	Mean	*115*	*75*	*96*	*172*	*1,884*
	S.E.	*±15.8*	*±15.8*	*±21.2*	*±20.5*	*±424.2*
	Range	*40–220*	*10–160*	*10–240*	*90–320*	*320–4,800*
			Normal boys			
50	7	30	10	60	70	640
51	7	20	10	20	30	160
52	12	60	50	80	130	480
53	12	40	20	5	25	240
54	12½	40	30	70	100	160
55	12	80	60	20	80	2,800
56	13	40	12	60	72	640
57*	15	60	32	240	272	6,400
	Mean	*46*	*28*	*69*	*97*	*1,440*
	S.E.	*±6.8*	*±6.7*	*±26.2*	*±27.7*	*±771.9*
	Range	*20–80*	*10–60*	*5–240*	*25–272*	*160–6,400*

X_4	$t=3.542$;	$p=0.01-0.001$
F	$t=2.433$;	$p=0.05-0.02$
Cortisone	$t=0.809$;	$p=0.5\ -0.4$
Cortisone+Comp. F	$t=2.209$;	$p=0.05-0.02$
Tetrahydrocortisone	$t=0.540$;	$p=0.6\ -0.5$

* Manifestations of puberty present.

X_4, were regularly present in the free fractions. Tetrahydrocortisone and tetrahydro-compound F were present in the conjugated fractions, the former in much greater amount than the latter. Semiquantitative estimations of each of these compounds, except tetrahydro-compound F, were made as described in the foregoing section.

Total neutral 17-ketosteroids were measured on 24-hour urine specimens of normal and adipose children, using a method similar to that recommended by the Medical Research Council committee on endocrinology (6). Fractionation of the 17-ketosteroid extracts into eight main constitu-

TABLE 3. THE EXCRETION OF 17-KETOSTEROIDS IN ADIPOSE AND NORMAL GIRLS (MG./24 HRS.)

Case No.	Age (yrs.)	Total 17-KS	β Fraction	Androsterone	Etiocholanolone	A/E ratio	11-Oxy 17-KS
			Adipose gynism				
11	14	9	0.8	3.9	2.5	1.6	1.4
12	14	7	1.5	2.7	2.5	1.1	0.4
13	13	4	1.0	1.0	1.0	1.0	1.0
14	14	5	1.1	1.6	1.6	1.0	0.9
15	12	2	0.6	0.8	0.5	1.6	0.4
16	15	2	0.3	1.0	0.4	2.5	0.3
Mean		*4.8*	*0.9*	*1.8*	*1.4*	*1.5*	*0.7*
S.E.		*±1.14*	*±0.17*	*±0.50*	*±0.38*	*±0.23*	*±0.18*
Range		*(2–9)*	*(0.3–1.5)*	*(0.8–3.9)*	*(0.4–2.5)*	*(1.0–2.5)*	*(0.3–1.4)*
			Normal girls				
7*	15	4	0.9	1.0	1.7	0.6	0.3
8*	15	5	1.2	1.7	1.6	1.1	0.6
9	12	2	0.5	0.7	0.6	1.2	0.2
10*	14	4	0.9	1.3	1.0	1.3	0.5
Mean		*3.8*	*0.9*	*1.2*	*1.2*	*1.1*	*0.4*
S.E.		*±0.63*	*±0.14*	*±0.21*	*±0.26*	*±0.16*	*±0.09*
Range		*(2–5)*	*(0.5–1.2)*	*(0.7–1.7)*	*(0.6–1.7)*	*(0.6–1.3)*	*(0.2–0.6)*

Total	$t=0.696$; $p=0.6-0.5$
β Fraction	$t=0$; $p=1$
Androsterone	$t=0.973$; $p=0.4-0.3$
Etiocholanolone	$t=0.383$; $p=0.8-0.7$
A/E ratio	$t=1.314$; $p=0.3-.20$
11-Oxy 17-ketosteroids	$t=1.420$; $p=0.2-0.1$

* Manifestations of puberty present.

TABLE 4. THE EXCRETION OF 17-KETOSTEROIDS IN ADIPOSE AND NORMAL BOYS (MG./24 HRS.)

Case No.	Age (yrs.)	Total 17-KS	β Fraction	Andro-sterone	Etiocho-lanolone	A/E ratio	11-Oxy 17-KS
				Adipose gynandrism			
29†	17	5	0.4	2.6	1.0	2.6	1.0
30	13	6	0.8	3.2	1.3	2.5	0.7
31	10	2	0.2	0.8	0.9	0.9	0.3
32	12	2	0.3	0.8	0.4	2.0	0.3
33	12	2	0.4	0.6	0.8	0.75	0.4
Mean		*3.4*	*0.4*	*1.6*	*0.9*	*1.8*	*0.5*
S.E.		*±0.87*	*±0.32*	*±0.54*	*±0.15*	*±0.39*	*±0.13*
Range		*(2–6)*	*(0.2–0.8)*	*(0.6–3.2)*	*(0.4–1.3)*	*(0.75–2.6)*	*(0.3–1.0)*
				Normal boys			
1*	14	9	0.6	1.1	0.9	1.2	0.4
2	11	2	0.1	0.6	0.4	1.5	0.7
3	9	1	0.1	0.2	0.2	1.0	0.3
4	11	1	0.1	0.3	0.2	1.5	0.4
5	11	1	0.1	0.2	0.2	1.0	0.4
6*	13	5	0.4	2.0	1.7	1.2	0.8
Mean		*3.2*	*0.2*	*0.7*	*0.6*	*1.2*	*0.5*
S.E.		*±1.33*	*±0.28*	*±0.29*	*±0.24*	*±0.09*	*±0.08*
Range		*(1–9)*	*(0.1–0.6)*	*(0.2–2.0)*	*(0.2–1.7)*	*(1–1.5)*	*(0.3–0.8)*

Total	$t=0.138$; $p=0.9-0.8$
β Fraction	$t=1.419$; $p=0.2-0.1$
Androsterone	$t=1.490$; $p=0.2-0.1$
Etiocholanolone	$t=0.092$; $p>0.9$
A/E ratio	$t=1.414$; $p=0.2-0.1$
11-Oxy 17-ketosteroids	$t=0$; $p=1.0$

* Manifestations of puberty present.

† Case 29 has been included, although the patient was 17 years old, because puberty was incomplete and the values are of the same order as those in the younger boys.

ents was carried out by the method of Pond (7), based on the more elaborate absorption technique of Dingemanse, Huis in't Veld and de Laat (8).

The results of the urinary adrenocortical steroid estimations in a series of 15 girls with adipose gynism and 8 normal girls are shown in Table 1; similar results in a series of 8 boys with adipose gynandrism and 8 normal boys are shown in Table 2. The results of 17-ketosteroid estimation and

fractionation of the urine in 5 boys with adipose gynandrism were compared with those in 6 normal boys; and in 6 girls with adipose gynism with those in 4 normal girls (Tables 3 and 4; Fig. 1).

DISCUSSION

Cortisone and compound F are glucocorticoids; that is, they are concerned in carbohydrate metabolism and when injected into adrenalectomized mice cause deposition of glycogen in the liver. It is not yet known whether compound X_4 is a glucocorticoid. Tetrahydrocortisone is a metabolic end-product of cortisone and compound F but, owing to the reduction of the Δ^4-3-ketone system in the molecule, it is not itself biologically active. The quantities of these substances excreted in the urine, therefore, are an indication of the production of glucocorticoids in the adrenal cortex, but it must be emphasized that they represent only a small fraction of the excreted adrenocortical steroids and their metabolites. In order to obtain a more accurate assessment of adrenocortical activity, all of these should be estimated.

Cortisone and compound F are mutually convertible in the human body (9) and it is therefore useful to consider the sum of these two substances.

Adipose gynism. Table 1 shows that there was a significantly increased excretion of compound F in the cases of adipose gynism; the increased excretions of cortisone, tetrahydrocortisone, X_4 and combined cortisone and compound F are of doubtful significance. Considered individually, Cases 13 and 22 showed excretions of both cortisone and compound F well outside the observed normal range. Three patients showed an excretion of compound F which appeared to be well outside the normal range, and 5 excreted more tetrahydrocortisone than any of the controls. It was impossible to correlate these individual results with the clinical condition of the patients.

Adipose gynandrism. Table 2 shows that in this condition there were increases in the excretion of compound F, cortisone and compound F combined, and X_4, which appeared significant. Seven of the patients with adipose gynandrism excreted compound F in larger amounts than any of the controls.

It is unfortunate that the number of normal controls was smaller than the number of patients.

A similar picture has been obtained in Cushing's syndrome, the average excretion of cortisone + compound F being greater in this condition than in a series of normal subjects, although in some individual cases the excretion of cortisone+compound F is not increased. It has been suggested that cases of Cushing's syndrome may show fluctuations in adrenal activity (10). Many workers, however, claim that the plasma concentrations of

17-hydroxycorticoids are consistently raised in Cushing's disease; but personal discussion with some of these workers reveals that repeated estimations of the plasma corticoid level are necessary in many cases before an increased concentration is observed. This would support the view that patients with Cushing's syndrome are subject to spontaneous fluctuations in the intensity of their disease. An alternative possibility, is suggested by the relatively normal level of excretion of tetrahydrocortisone, which would indicate that in some cases there is a delayed inactivation of the active hormones. Hypercorticoidism would then result from an increased concentration of corticoids in the plasma, due to delay in inactivation associated with a normal total rate of production. Perhaps even Cushing's syndrome may be associated with heightened sensitivity of the tissues to relatively normal concentrations of circulating hormones.

It is not impossible, therefore, that adipose gynism and gynandrism may also be associated with spontaneous fluctuations in adrenal activity, or to decreased rates of inactivation of the active hormones, or even to heightened sensitivity of the tissues to normal concentrations.

In our series the excretion of total 17-ketosteroids in adipose gynism and in gynandrism was within the same range as that in normal children. Although fractionation of the 17-ketosteroids on alumina yielded eight fractions, each of which was identified by the addition of known steroids and more recently by paper chromatography (13), the results obtained from analysis of the first three were summed to give the β fraction. Fractions IV and V consisted mainly of the biologically active androsterone and its inactive isomer, etiocholanolone, respectively. Fractions VI and VII were also summed to give the 11-oxy 17-ketosteroids. The eighth fraction was of unknown nature, and may have consisted of impurities; it was not included in the tables.

Statistical analysis shows that the differences between the results in adipose gynism and gynandrism do not vary significantly from the differences observed in normal girls and boys, respectively. Pond (11) has shown that in Cushing's syndrome excretion of the 11-oxy 17-ketosteroids is increased above normal. Only in 2 of the 11 cases of adipose gynism and gynandrism was an increase apparent. Although there may be an altered pathway of metabolism of the 11-oxygenated adrenal steroids in childhood, it is much more likely that the quantities of the steroids excreted in childhood are approaching the limits of sensitivity of the method. In 4 individual cases the value for the 11-oxy 17-ketosteroid fraction was outside the normal range for children but not outside that for adults. In only 2 of these, however, was there a corresponding increase in the excretion of cortisone or compound F. It was not possible for the two types of analysis to be carried out on the same specimen of urine. These studies

show that in some cases there may be an increased activity of the adrenal cortex in adipose gynism and gynandrism. This is not regularly present and it remains an open question as to whether this heightened activity is coincidental, or whether there is a causal relationship to heightened adrenal activity—in which case that activity would be intermittent, as in Cushing's syndrome.

The mean androsterone/etiocholanolone ratios in the two groups of patients was not significantly different from the ratios in the corresponding controls. In 3 of the girls with adipose gynism and 3 of the boys with adipose gynandrism the ratios were above the normal range. It is tempting to speculate that this increase may be related to the increased stature of the children, especially since the A/E ratio in all the children studied was greater than is found in normal adults. However, although androsterone is a growth hormone in that it is a protein anabolic agent, the absolute quantities excreted are less than in the adult.

The increased plasma corticotropin levels observed by Parrott (12) in some of these children are of interest. The children show several features allied to those of Cushing's syndrome, *e.g.*, plethoric facies, acrocyanosis, red or purple lineae distensae, larger breasts with distinctive areolae, and similar type of adiposity. However, they differ in so far as their height is above normal rather than below normal. Moderate dwarfism frequently occurs in children with true Cushing's syndrome. The children show neither osteoporosis, nor diabetes mellitus. Simpson (1, 3) has postulated an excess of growth hormone and possibly an excess of islet-cell secretion and insulin.

SUMMARY

1. The excretion of adrenocortical steroids and of 17-ketosteroids has been estimated in a series of normal and adipose children.

2. In adipose gynism and in adipose gynandrism the excretion of compound F was significantly increased above that of normal children. In children with adipose gynandrism the excretion of an unknown Δ^4-3-ketosteroid, X_4, was also significantly increased.

3. There was no significant difference in the excretions of 17-ketosteroid fractions between adipose and normal children.

Note added in proof

Compound X_4 has recently been shown (Holness, 1956) to be Reichstein's compound U (Δ^4-pregnene-17α, 20β-21 triol-3,11-dione).

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EFFECT OF ANDROGENS ON PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS*

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THE hyperadrenocorticism observed in patients following large doses of cortisone resembles in many respects that seen in Cushing's syndrome. Albright (1) found androgens to be of some value in correcting many of the manifestations of this disease. Pearson and Eliel (2) also reported that testosterone would counteract some of the effects of cortisone on normal tissue. In animal experiments androgens have been shown to possess a sparing action upon the adrenal cortices of animals treated with cortisone (3, 4).

Butler *et al.* (5) gave testosterone to 2 adrenalectomized patients maintained on 25 to 50 mg. of cortisone daily. Within twenty-four hours the adrenocortical requirement increased and an Addisonian crisis was precipitated. The situation was controlled by increasing the cortisone dosage.

This paper deals with the effect of androgens on the plasma levels of 17-hydroxycorticosteroids (17-OH-CS) in unstressed patients and in patients stressed by surgical operation.

MATERIAL AND METHODS

Thirty-four patients from the Radiumhemmet at Karolinska Sjukhuset were selected at random. The average age of the patients was 53 years (15–77 years). Eighteen had cancer and the rest had nonmalignant tumors. In addition 2 adrenalectomized patients (IN and AK) were investigated.

The androgens (testosterone propionate, testosterone phenylpropionate and methyl androstenediol) in doses of 50 or 100 mg. were injected intramuscularly daily during one to twelve days.

Blood samples for estimation of 17-OH-CS were taken from 7 unstressed patients before and after treatment with the androgens. Immediately after the blood samples were taken, each patient received a single intramuscular injection of 20 I.U. of corticotropin (ACTH) and additional blood samples were drawn two and four hours later.

Of 27 patients operated upon, 16 were pretreated with daily injections of 50 to 100 mg. of the androgens, and 11 were used as controls. Blood samples for determination of 17-OH-CS were taken immediately after the operation and twenty-four hours later.

The urinary excretion of corticosteriods was estimated before and after treatment with androgens in the 2 adrenalectomized patients maintained on 50 to 100 mg. of cortisone daily.

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The 17-OH-CS level in blood was determined according to the method of Nelson and Samuels (6), using 10-ml. samples of plasma. The chromatography was carried out on magnesium silicate-Celite columns. Correction for background absorption was made by using the color correction equation described by Allen (7).

The 17-hydroxycorticosteroids are expressed in micrograms per 100 milliliters of plasma. The mean plasma level of 22 healthy persons at 8 a.m. was found to be 8.5 ± 1.41 μg. per 100 ml., and the difference between duplicates was ±12 per cent.

The corticosteroids in the urine were estimated by Dr. E. Diczfalusy, Stockholm, using a modification of the Norumberski method (8).

The 17-ketosteroids in the urine were determined by a method described by Callow, Callow and Emmens, modified by Vestergaard (9).

TABLE 1. THE EFFECT OF TESTOSTERONE PROPIONATE ON THE PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS IN UNSTRESSED PATIENTS BEFORE AND AFTER STIMULATION WITH CORTICOTROPIN (ACTH)

Treatment	No. of patients	Plasma levels of 17-OH-CS (μg./100 ml.)		
		Before ACTH-stimulation	After ACTH-stimulation	
			2 hours	4 hours
None	7	8.1±2.03*	20.0±4.74	20.1±2.79
Testosterone propionate, 50 mg. daily for 3–4 days	7	9.0±1.28	19.3±3.26	22.5±3.07

* Mean and standard deviation.

RESULTS

Plasma levels of 17-hydroxycorticosteroids

Before and after treatment with androgens. No significant difference was found in the plasma levels of 17-OH-CS in 7 patients before and after treatment with testosterone propionate in a dosage of 50 mg. daily for three to four days (Table 1).

Effect of corticotropin (ACTH) on — in patients treated with androgens. No significant difference was found between the effect of ACTH on the plasma levels of 17-OH-CS in 7 patients pretreated with 50 mg. of testosterone propionate daily for three to four days and the effect in 7 untreated patients (Table 1).

Effect of operation on — in patients treated preoperatively with androgens. Testosterone propionate, testosterone phenylpropionate and methyl androstenediol in dosages of 50 or 100 mg. administered daily for two to six days prior to a major surgical operation prevented the usual increase in the plasma steroid level (10). Testosterone propionate in a single dose of 100 mg. the day before the operation had no effect (Table 2).

TABLE 2. THE EFFECT OF VARIOUS ANDROGENS ON THE PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS IN PATIENTS STRESSED BY MAJOR SURGICAL OPERATIONS

Treatment	No. of patients	Plasma level of 17-OH-CS (μg./100 ml.)		
		Before operation	After operation	
			1 hour	24 hours
None	11	5.6±0.93*	31.4±1.91	29.5±2.72
Testosterone propionate, 50 mg. daily for 4–6 days	5	7.1±1.42	7.0±3.10	3.8±1.21
Testosterone phenylpropionate, 50 mg. daily for 2–3 days	4	7.5±1.49	9.3±2.64	7.6±3.39
Methylandrostenediol, 100 mg. daily for 3–4 days	6	7.0±1.38	5.2±2.11	8.1±3.14
Testosterone propionate, 100 mg. in 1 day	1	4.5	21.0	26.0

* Mean and standard deviation.

Urinary excretion of corticosteroids and 17-ketosteroids

In 2 adrenalectomized patients treated with daily injections of testosterone propionate or methyl androstenediol. (Patient IN was maintained on 100 mg., and Patient AK on 50 mg. of cortisone daily in tablet form.) Testosterone propionate in a dosage of 50 mg. daily for three days caused a decrease in the urinary corticosteroids from about 35 mg. per twenty-four hours to 13 mg. Two days after the last injection of testosterone propionate the urinary corticosteroids had returned to the pre-injection values (Fig. 1). Methyl androstenediol in a dosage of 100 mg. daily for four

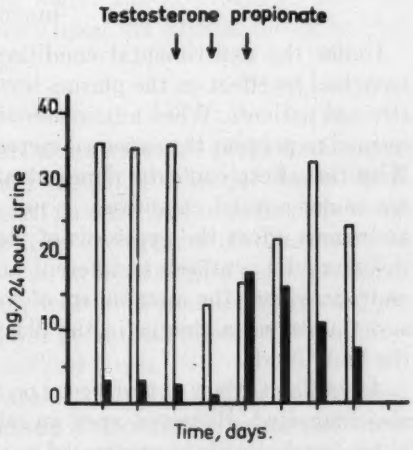

FIG. 1. The effect of 3 injections of 50 mg. of testosterone propionate on the urinary excretion of corticosteroids (white columns) and 17-ketosteroids (black columns) in an adrenalectomized patient.

days caused a decrease in the urinary corticosteroids, the lowest level being reached three days after the last injection. On the fourth day, the urinary corticosteroids started to increase again (Fig. 2).

The urinary 17-ketosteroids increased very markedly after the administration of testosterone propionate. After methyl androstenediol, however, no change could be demonstrated (Figs. 1 and 2).

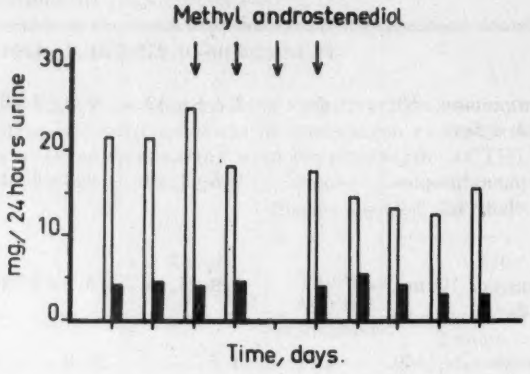

FIG. 2. The effect of 4 injections of 100 mg. of methyl androstenediol on the urinary excretion of corticosteroids (white columns) and 17-ketosteroids (black columns) in an adrenalectomized patient.

DISCUSSION

Under the experimental conditions in this study, androgens seem to have had no effect on the plasma levels of 17-hydroxycorticosteroids in unstressed patients. When administered preceding stress, however, androgens seemed to prevent the expected increase in the plasma corticosteroid levels. Why this effect could be demonstrated only in patients under stress and not under normal conditions, is not clear. It may be due to the fact that androgens affect the synthesis of steroids in the adrenal cortex and that this steroidal synthesis is different under stress. Another possibility is that androgens alter the metabolism of cortical steroids in the peripheral tissue and thus cause a change in the disappearance rate of these steroids from the body fluids.

An indirect effect of androgens on the adrenal cortex via the pituitary is also suggested. However, such an inhibitory effect on the pituitary ought to be demonstrable in unstressed as well as in stressed patients.

A direct effect of androgens on the adrenal cortex, affecting steroidal synthesis, may change the cortical response to stimulation by corticotropin. The results of this study show, however, that corticotropin has

the same effect on the plasma steroids, whether it be given before or after the administration of androgen.

In adrenalectomized patients treated with cortisone, administration of androgens results in a decrease in the urinary excretion of steroid metabolites originating from exogenous cortisone. Since in these cases the androgens have no effect on the synthesis of the steroids of the adrenal cortex (and, due to the lack of a target organ, the effect of pituitary corticotropin may be excluded), their effect may be accounted for by a change in the metabolism of cortical steroids in the peripheral tissue. The mechanism involved will be further investigated.

In adrenalectomized Patient IN, maintained on 100 mg. of cortisone daily, administration of 150 mg. of testosterone propionate caused no symptoms of adrenal insufficiency. In adrenalectomized Patient AK, however, the administration of 400 mg. of methyl androstenediol caused severe symptoms of adrenocortical insufficiency with fever, nausea and weakness. The data are too few to allow any conclusions about the amount of cortisone necessary to "neutralize" androgens, but it may be suggested that in order to avoid an Addisonian crisis in adrenalectomized patients treated with androgens, large daily doses of cortisone are necessary. A dosage of 50 mg. of cortisone a day was not enough to neutralize the effect of 400 mg. of methyl androstenediol.

The results of this study indicate that the effect of androgens on the plasma levels of 17-hydroxycorticosteroids may be due to a change in the metabolism of these steroids in the body. The investigation does not furnish evidence that the effect is directly upon the adrenal cortex, or that it is mediated by pituitary corticotropin.

SUMMARY

A study has been made of the effects of testosterone propionate, testosterone phenylpropionate or methyl androstenediol 1) on the plasma levels of 17-hydroxycorticosteroids in unstressed patients and in patients stressed by surgical operation, and 2) on the urinary excretion of corticosteroids in 2 adrenalectomized patients maintained on 50 to 100 mg. of cortisone daily.

No effect was found on the plasma corticosteroid levels in unstressed patients. Administered preceding a surgical operation, however, the androgens prevented the expected increase in these levels.

In 2 adrenalectomized patients, maintained on 50 or 100 mg. of cortisone daily, administration of androgens caused a decrease in urinary corticosteroids and precipitated an Addisonian crisis in one of the patients.

It is suggested that the effect of androgens on the plasma levels of 17-hydroxycorticosteroids is due to a change in the metabolism of these steroids in the body.

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THE PERSISTENCE OF INSULIN SENSITIVITY IN ACUTE ADRENOCORTICAL HYPERACTIVITY INDUCED BY CORTICOTROPIN IN NORMAL MAN*

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INTRODUCTION

THERE are conflicting views concerning the effects of adrenocortical hyperactivity on the blood glucose response to insulin. A decreased response has been described in Cushing's disease (1), during administration of corticotropin in normal man (2), and in chronically ill patients given cortisone (3). On the contrary, no change in response has been reported in normal subjects given cortisone (3), or in chronically ill patients receiving corticotropin (4).

Because of variation of the foregoing experimental methods and observations, the studies here reported were conducted in order to determine the effect of insulin on the blood glucose concentration in normal man during intense acute adrenocortical hyperactivity induced by intravenous infusion of corticotropin (ACTH).

METHODS

The subjects were 8 normal medical personnel of average weight and aged from 23 to 37 years. Although constant diets were not prescribed prior to testing, it was felt that their diets were sufficiently uniform to eliminate any effects of variable carbohydrate intake on insulin action.

Insulin tolerance tests were carried out in a control state, and in a state of acute adrenocortical hyperactivity induced by intravenous infusion of ACTH in a dosage intended to provoke intense stimulation (5).

In the control state the subjects rested and fasted for fourteen hours prior to testing. In the corticotropin experiments the conditions were similar, except that during the eight hours preceding the testing a dose of 20 mg. of ACTH in physiologic saline was administered by constant intravenous infusion, and an additional dose of 5 mg. was given during the

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TABLE 1. THE BLOOD GLUCOSE AND SERUM PHOSPHORUS AND POTASSIUM CONCENTRATIONS AFTER INTRAVENOUS INSULIN IN THE CONTROL STATE

Subject	Minutes after injection of Insulin					
	0	30	60	90	120	180
	Blood glucose (mg./100 cc.)					
HK	93	39	75	84	86	89
LR	87	24	60	74	86	87
HS	86	30	54	71	78	83
JM	88	49	65	68	70	77
WR	84	30	65	73	80	81
MB	88	24	55	59	68	89
JH	83	34	54	69	75	77
AM	93	41	64	72	86	87
Mean	*87.6*	*33.9*	*61.5*	*71.2*	*78.6*	*83.6*
SD±	*3.6*	*9.0*	*7.2*	*6.8*	*7.3*	*5.1*
	Serum phosphorus (mg./100 cc.)					
HK	2.7	1.3	1.3	1.8	2.1	2.3
LR	4.2	2.4	2.3	2.9	3.0	3.3
HS	4.0	3.4	2.8	3.0	2.9	3.4
JM	3.1	1.4	1.6	2.1	2.4	2.4
WR	4.1	3.0	2.9	3.3	3.8	3.5
MB	4.2	2.3	2.1	2.7	3.0	2.9
JH	3.5	2.2	2.1	2.7	2.9	3.0
AM	3.4	2.3	2.2	2.7	2.7	3.1
Mean	*3.63*	*2.28*	*2.16*	*2.66*	*2.84*	*2.96*
SD±	*0.6*	*0.7*	*0.5*	*0.5*	*0.5*	*0.4*
	Serum potassium (mEq./L)					
HK	4.5	4.3	—	4.1	4.0	4.7
LR	3.8	3.2	3.1	3.9	4.4	4.7
HS	4.3	3.8	3.5	3.9	4.3	4.8
JM	3.4	3.1	3.0	3.1	3.5	3.5
WR	4.1	3.6	3.6	4.8	4.0	4.4
MB	4.7	3.6	3.2	4.0	4.4	4.9
JH	4.3	3.9	—	4.8	5.0	5.0
AM	5.2	3.9	3.6	4.2	5.0	5.3
Mean	*4.28*	*3.66*	*3.32*	*4.11*	*4.33*	*4.67*
SD±	*0.5*	*0.4*	*0.2*	*0.5*	*0.5*	*0.5*

TABLE 2. THE BLOOD GLUCOSE AND SERUM PHOSPHORUS AND POTASSIUM CONCENTRATIONS AFTER INTRAVENOUS INSULIN DURING ADRENOCORTICAL HYPERACTIVITY

Subject	Minutes after injection of Insulin						
	−8 hrs.	0	30	60	90	120	180
	Blood glucose (mg./100 cc.)						
HK	94	113	82	84	99	100	102
LR	86	109	68	67	74	83	91
HS	85	98	42	70	82	93	98
JM	82	113	70	86	98	99	99
WR	86	104	57	85	88	93	93
MB	92	123	84	81	98	103	110
JH	89	126	58	87	96	107	105
AM	93	121	69	71	87	94	95
Mean	*88.3*	*113.4*	*66.1*	*78.9*	*90.2*	*96.4*	*99.0*
SD±	*4.2*	*9.6*	*13.8*	*8.0*	*9.0*	*7.4*	*6.5*
	Serum phosphorus (mg./100 cc.)						
HK	3.9	2.1	1.2	1.6	1.7	1.8	2.0
LR	4.3	4.1	3.3	3.9	4.1	4.6	4.6
HS	4.7	3.1	2.6	2.4	3.3	3.5	3.5
JM	3.2	2.3	1.4	1.9	2.3	2.2	2.8
WR	4.0	3.3	2.3	2.3	2.7	2.7	3.3
MB	3.7	2.4	1.8	1.8	1.8	1.4	2.0
JH	3.8	2.4	2.4	2.8	2.7	2.4	2.5
AM	3.4	2.2	1.8	1.8	1.7	2.0	2.0
Mean	*3.85*	*2.74*	*2.10*	*2.30*	*2.53*	*2.59*	*2.86*
SD±	*0.5*	*0.7*	*0.7*	*0.8*	*0.8*	*1.1*	*0.9*
	Serum potassium (mEq./L)						
HK	4.7	4.8	4.1	4.8	4.4	5.1	4.7
LR	3.8	4.0	3.8	4.5	4.4	4.4	4.2
HS	4.2	4.1	3.8	—	4.1	4.0	3.5
JM	3.5	4.2	3.8	4.0	4.4	4.5	4.4
WR	4.2	4.7	4.7	—	4.9	5.6	4.8
MB	4.2	4.2	3.8	4.1	4.2	4.4	4.5
JH	4.1	5.6	4.1	4.7	4.8	4.8	4.9
AM	4.2	5.3	4.0	4.2	4.4	5.1	4.6
Mean	*4.11*	*4.61*	*3.99*	*4.39*	*4.46*	*4.73*	*4.43*
SD±	*0.3*	*0.6*	*0.3*	*0.3*	*0.3*	*0.5*	*0.4*

following three hours, in order to maintain stimulation during the insulin tolerance tests.

In the corticotropin experiments a mean fall of eosinophils (6) from 254 to 0 cells per cu. mm. of whole blood during the eight hours of preliminary adrenocortical stimulation, and a mean rise of 24-hour urinary 17-ketosteroid (7) content from 19.9 mg. (control) to 26.5 mg. (during and following corticotropin) were interpreted as evidence of adrenocortical hyperactivity (8).

Insulin, free of hyperglycemic factor,[1] was injected intravenously (0.1 unit per Kg. body weight) and blood samples were collected at 0, 30, 60, 90, 120, and 180 minutes for determination of the concentrations of blood glucose (9), serum phosphorus (10) and serum potassium by flame photometry.

RESULTS

In Tables 1 and 2 are given the values obtained in the control and in the corticotropin experiments respectively. In Figure 1 the data are plotted to

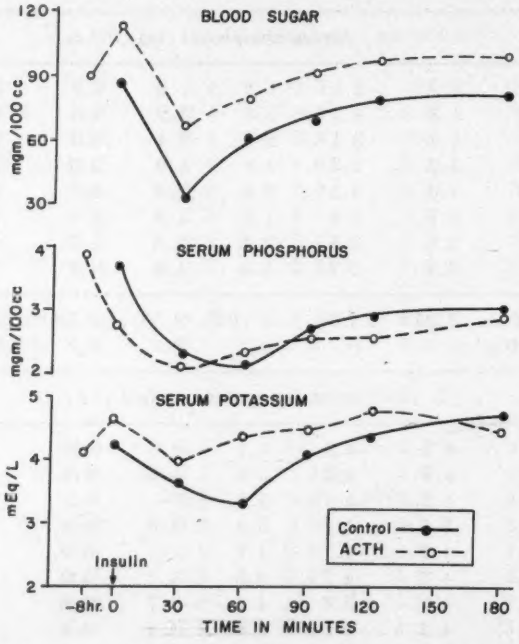

FIG. 1. The mean actual concentrations of blood glucose and serum potassium and phosphorus after injection of insulin.

[1] Supplied through the courtesy of Dr. W. R. Kirtley, Eli Lilly and Company, Indianapolis.

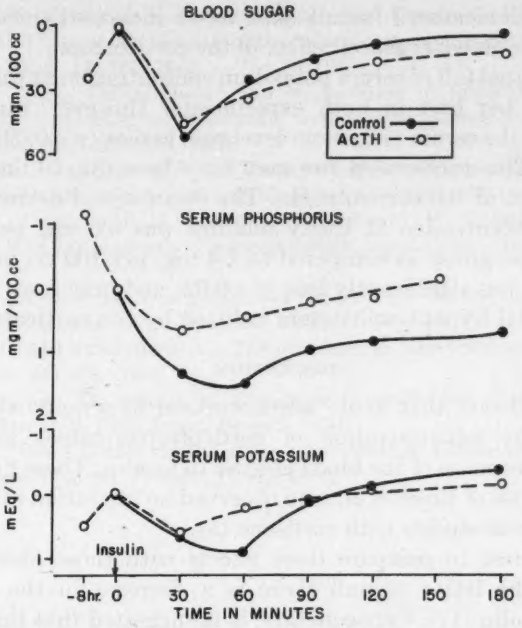

FIG. 2. The changes in blood glucose and serum potassium and phosphorus concentrations after injection of insulin, expressed as mean deviations from the values of the initial samples.

show mean actual values, and in Figure 2 to show mean deviations from the initial value for the sample drawn at the time of insulin injection.

The preliminary administration of ACTH brought about a mean rise of 25 mg. per 100 cc. in the blood sugar level. This proportionately higher level was maintained throughout the test (Fig. 1). In addition, following administration of ACTH there was an increase in the concentration of serum potassium and a decrease in that of the serum phosphorus. However, these conditioned levels were not maintained.

Thirty minutes following the injection of insulin the blood glucose concentration decreased by 54 mg. per 100 cc. in the control, and by 47 mg. per 100 cc. in the ACTH experiments. There were no significant differences between these values and those at other equivalent points on the mean curves (Fig. 2). Therefore, though corticotropin brought about a rise in blood sugar concentration, it caused no change in the actual response to injected insulin.

Three subjects experienced symptoms of hypoglycemia in the control tests. However, no such manifestations were noted during adrenocortical stimulation. The absence of the symptoms of hypoglycemic response in the latter experiments may have been due to the actual higher levels of blood

glucose, or to unmeasured factors such as an increased turnover rate of glucose from the gluconeogenetic effect of the corticotropin.

The mean actual fall of serum potassium concentration at thirty minutes was 0.6 mEq. per liter in both experiments. However, during ACTH administration the serum potassium level rose earlier ($p < 0.02$) than in the control state. This accelerated rise may have been due to the potassium-mobilizing effect of the corticotropin. The mean actual decrease of serum phosphorus concentration at thirty minutes was 0.6 mg. per 100 cc. in the corticotropin group as compared to 1.4 mg. per 100 cc. in the control state. This fall was significantly less ($p < 0.02$) and may have been in part due to the initial hypophosphatemia induced by the corticotropin.

DISCUSSION

The data indicate that acute adrenocortical hyperactivity induced in normal man by administration of corticotropin causes no significant change in the response of the blood glucose to insulin. These findings are in accord with those of Engel *et al.* who observed no alteration in the response to insulin in acute studies with cortisone (3).

It is of interest to compare these effects with those observed during stress. With the latter stimuli there is a decrease in the blood sugar response to insulin (11). Consequently, it is suggested that this decrease is mediated by other factors, working alone or in conjunction with adrenocortical hyperactivity. These findings, then, offer further evidence for the concept advanced by Ingle that the metabolic response to acute stress is not wholly accounted for by adrenocortical hyperactivity (12).

SUMMARY

The acute adrenocortical hyperactivity induced by administration of corticotropin to normal man does not affect the response of the blood glucose to insulin, as measured by the insulin tolerance test.

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SUPPRESSION OF UTERINE GROWTH BY PURIFIED HYDROCORTISONE ACETATE, 9α-FLUOROHYDROCORTISONE ACETATE AND CORTICOTROPIN*†

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CERTAIN recent studies have emphasized interactions that may occur between hormones from the adenohypophysis, suprarenal cortex and ovary to modify the responses of target organs of the female reproductive system (1, 2). It is becoming increasingly apparent that these responses are not the result of the independent action of one or perhaps two hormones only. It is more likely that they represent the physiologic expression of the sum total of all present biologic hormones and their metabolites, acting in concert on the receptor-organs (3–5).

The present work deals with interactions between hydrocortisone acetate or 9α-fluorohydrocortisone acetate and estradiol-17β. As in other studies (6), we used the uterus of the ovariectomized rat as a test organ. In order to clarify the effects obtained, we also utilized purified corticotropin (ACTH) in similarly prepared animals and also in those adrenalectomized as well as ovariectomized.

MATERIALS AND METHODS

Virgin albino arts 100 days of age and weighing approximately 200 grams were used for these experiments. They were bilaterally ovariectomized, and treatment was started seven days later. Estradiol-17β was dissolved in sesame oil, and hydrocortisone acetate[1] and 9α-fluorohydrocortisone acetate[2] were dissolved in an aqueous vehicle containing 0.9 per

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[1] The hydrocortisone acetate was obtained through the generosity of Merck and Company.

[2] We are grateful to E. R. Squibb and Sons for the 9α-fluorohydrocortisone acetate.

cent benzyl alcohol, 3 drops of Tween 60, and 0.9 per cent sodium chloride. The corticotropin[3] used in these studies was injected in the form of ACTHAR-gel. All injections were administered once daily subcutaneously. When two substances were administered, they were injected at separate sites. Necropsies were performed seventy-two hours after the initial injection and the uteri and adrenals were carefully removed and weighed to the nearest 0.2 mg. on a Roller-Smith torsion balance. Twenty-four hours later the dry solid content of each uterus was determined.

RESULTS

1. *Control experiments*

Since the test substances were contained in different vehicles, it was important first to record the uterine growth-modifying property of each

TABLE 1. CONTROL EXPERIMENTS

The influence of aqueous vehicle, sesame oil, and pregnane 3α,20α-diol on uterine growth of ovariectomized rats

Treatment	No. of rats	Wt. of uterus (mg.)		Wt. of adrenals (mg.), wet
		wet	dry	
Ovariectomized controls	25	115.5±3.3*	22.4±0.7	59.9±2.0
0.1 cc. Sesame oil	15	116.4±2.4	22.8±0.8	60.2±2.2
0.1 μg. Estradiol-17β	36	227.3±6.1	42.3±1.8	59.4±1.5
0.1 μg. Estradiol-17β+0.2 cc. aqueous vehicle	15	230.4±4.2	42.8±1.6	60.4±1.6
0.1 μg. Estradiol-17β+6.0 Gm. pregnane 3α,20α-diol in aqueous vehicle	14	226.4±7.0	41.8±2.0	61.2±1.4

* Average ± Standard Error.

vehicle. Unfortunately the substrate of the ACTHAR-gel was not available without hormone. It was also necessary to establish that the addition of any inert steroidal substance would not interfere with the uterine growth induced by estradiol-17β.

The results of these control experiments are summarized in Table 1. It is apparent that sesame oil alone was without effect in promoting growth of the uterus in 15 ovariectomized rats. When 0.2 cc. of aqueous vehicle was given simultaneously with estradiol-17β to 15 rats, there was no modification of the uterine response. When 6.0 mg. of an "inert" steroid, pregnane

[3] We are indebted to Dr. Irby Bunding of Armour and Company for the generous amount of ACTHAR-gel given to us for this investigation.

3α,20α-diol in aqueous vehicle was given simultaneously with estradiol-17β to 14 rats, there was also no modification of the uterine growth pattern.

2. *Effect of estradiol-17β on uterine growth*

Previously, Hisaw, Velardo and Goolsby (7) determined the activity of several estrogens in promoting the growth of the uterus of the ovariectomized rat. In their studies they used albino rats derived from a highly inbred (Charles River) strain at the Harvard Biological Laboratories.

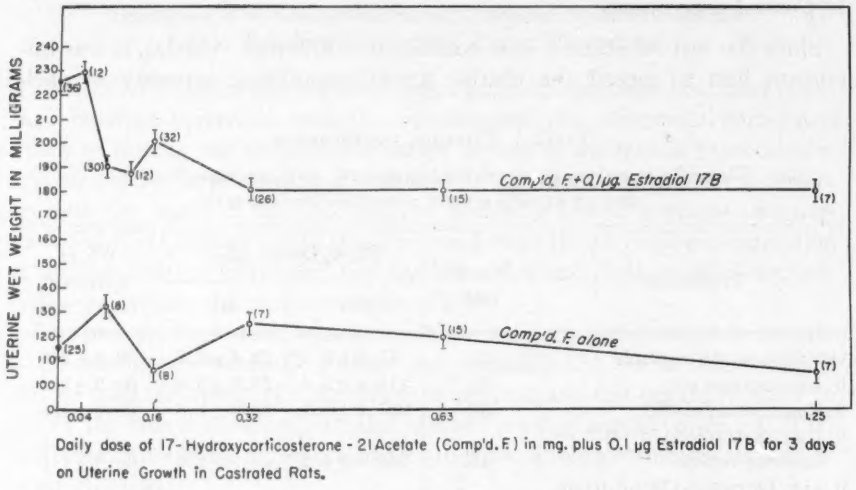

FIG. 1. Response (wet weight) of the uteri of ovariectomized rats to compound F acetate alone (lower line) and to a combination of compound F acetate plus 0.1 μg. of estradiol—17β (upper line). The figures in parentheses indicate the number of animals on each point.

Employing the foregoing procedures they found that the injection of 0.1 μg. of estradiol-17β daily for three days was correlated with the steepest rise on the dose-response curve. Such injections given one week after ovariectomy resulted in uteri that were 88 per cent heavier than those from non-treated, ovariectomized controls. In the present investigation, albino rats from the Charles River strain were used, and an increase of 94 per cent was observed. Therefore, for the following experiments, 0.1 μg. of estradiol-17β was chosen as the standard dosage of estrogen.

Throughout the text the term "growth" is used; it should be pointed out that growth data are based on both wet and dry uterine weights. On previous occasions nitrogen determinations were made on each group of uteri from the animals on each treatment schedule, and it was found that

the results for the nitrogen assays paralleled the curves for the dry weights. Therefore, in order to make the term "growth" more meaningful, it should be kept in mind that the nitrogen data follow the curves for the dry weights in a precise manner.

3. *Effect of hydrocortisone acetate on estradiol-induced uterine growth*

The activity of hydrocortisone acetate (compound F) alone on the uterus of the castrated rat was first studied. Doses of from 0.04 to 1.25 mg. were

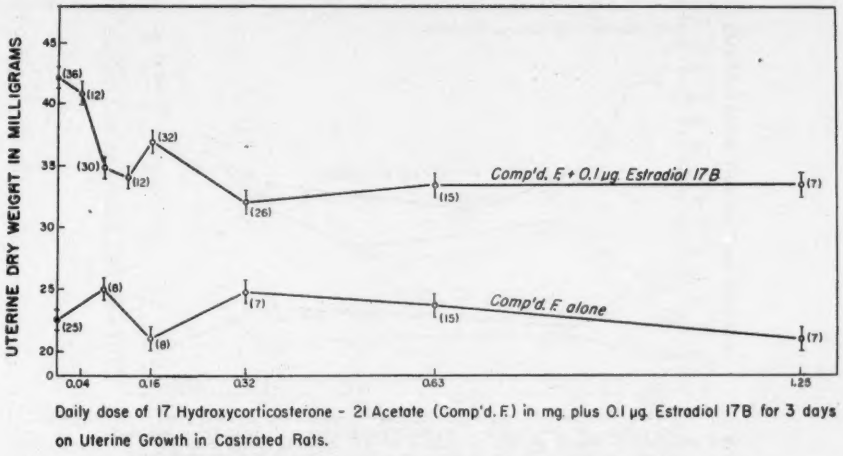

FIG. 2. Response (dry weight) of the corresponding uteri represented in Figure 1. Note the similarity of the curves showing the restriction of estradiol-induced uterine growth by compound F acetate.

used, and in Figures 1 and 2 it is seen that this substance alone was without effect in the promotion of uterine growth. Additional groups of animals receiving dosages between 1.25 and 5.0 mg. showed no significant departure from the arithmetical mean of the nontreated, ovariectomized controls.

In the groups of rats receiving a combination of compound F acetate and estradiol, a suppression of estradiol-induced uterine growth was observed with as little as 0.08 mg. of the adrenal hormone (Figs. 1 and 2). No further restriction of uterine growth occurred when more than 0.32 mg. of compound F was administered, and doses up to 5.0 mg. daily for three days effected no greater inhibition than that depicted in Figures 1 and 2.

4. *Effect of 9α-fluorohydrocortisone acetate on estradiol-induced uterine growth*

When administered alone, 9α-fluorohydrocortisone (9α-Fl compound F) acetate had no consistent or significant effect on either the wet or dry

weights of the uteri of ovariectomized rats (Figs. 3 and 4). On the other hand, it was found to be a potent compound in the suppression of estradiol-induced uterine growth. A significant decrease could be obtained with as little as 0.025 mg., on both wet-weight and dry-weight determinations. There seemed to be no significant difference in the restriction of the growth process between the 0.10, 0.20, and 0.40 mg. levels. When the daily

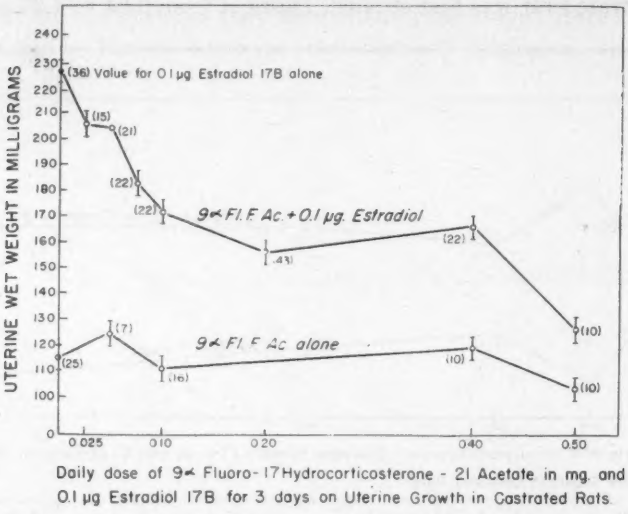

FIG. 3. The effects (wet weight) on the uteri of ovariectomized rats of 9α-Fl compound F acetate administered alone (lower line), and with 0.1 µg. of estradiol (upper line).

dosage of 9α-Fl compound F was increased to 0.50 mg., however, a further pronounced reduction in uterine growth was observed (Figs. 3 and 4). It is of particular significance that, although this compound markedly restricted the response of the uterus to estradiol, no undesirable side effects were observed. The animals maintained their body weight in a manner comparable to that in the group receiving estradiol alone or in the control group receiving the vehicle only.

That this substance is a potent glucocorticoid is further substantiated by its ability to reduce the weight of the adrenal glands. The arithmetical mean for the wet weight of the control adrenal glands was 60.0 mg., whereas this mean was only 44.8 mg. for the adrenals of rats receiving the higher dosages of 9α-Fl compound F acetate (0.40 to 0.50 mg. daily).

5. *Effect of purified corticotropin on estradiol-induced uterine growth*

The fact that purified compound F acetate and 9α-Fl compound F acetate were both active in suppressing the action of estradiol on uterine

growth made it seem worthwhile to determine the effects of a highly potent and purified preparation of corticotropin on this response. The data clearly indicate that when as little as 0.025 mg. of corticotropin (ACTHAR-gel) was administered concurrently with 0.1 μg. of estradiol-17β, the growth response was significantly suppressed (Figs. 5 and 6). It appears that 0.10 mg. of corticotropin induced the greatest decrease in the dry-weight values, whereas 0.40 mg. seem to be required for the greatest

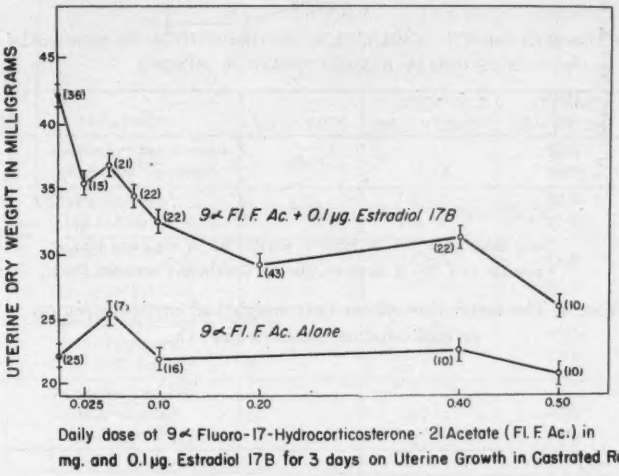

FIG. 4. The effects (dry weight) on the corresponding uteri represented in Figure 3. The wet-weight curve and the dry-weight curve parallel one another very closely.

decrease on the wet-weight basis. In general, however, there was good agreement between the curves depicting wet and dry weights (Figs. 5 and 6).

The adrenal weights of the animals receiving corticotropin were all definitely increased. In the corticotropin-estrogen treated rats the adrenals were at least 10 mg. heavier with the intermediate dosages of corticotropin than were the adrenals of the estrogen-treated group.

6. *Mechanism of the inhibition by corticotropin of estradiol-induced uterine growth*

In an effort to ascertain the mechanism of this inhibition the ovariectomized-adrenalectomized rat was used as a test animal. Bilateral adrenalectomy was performed one hour before the administration of the hormones; all other procedures were adhered to precisely as described previously. As shown in Figures 5 and 6, it was clearly established that when 0.5 mg. of corticotropin was administered concurrently (but at a separate

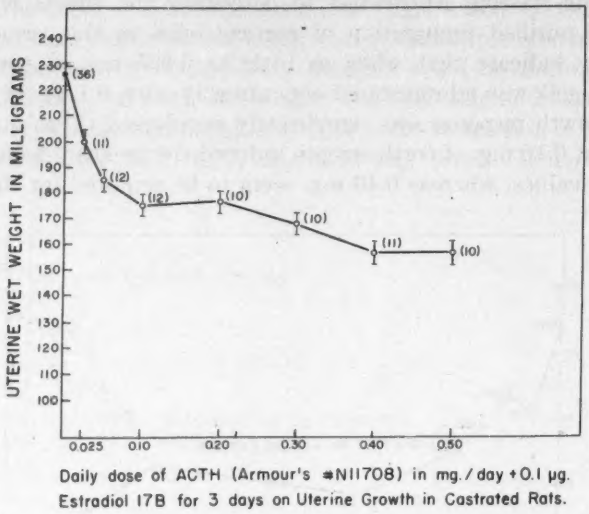

FIG. 5. The restrictive effects (wet weight) of corticotropin on estradiol-induced uterine growth.

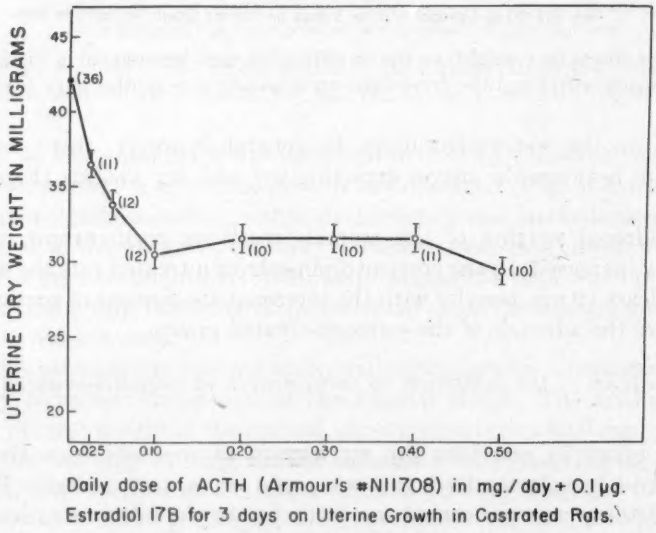

FIG. 6. The restrictive effects (dry weight) of corticotropin on the corresponding uteri represented in Figure 5.

injection site) with 0.1 μg. of estradiol-17β, the response of the uterus to the estrogen was markedly suppressed. When the same substances were given to animals that were bilaterally adrenalectomized as well as ovariectomized, the response of the uterus to the estrogen was not altered (Table 2). From these data, it seems justifiable to infer that the mechanism of the inhibition by corticotropin of estrogen-induced uterine growth is mediated by the adrenal glands.

TABLE 2

MECHANISM OF ACTH INHIBITION OF ESTRADIOL-17B INDUCED UTERINE GROWTH IN OVARIECTOMIZED, ALBINO RATS

TREATMENT	NO OF RATS	UTERINE WET WEIGHT	UTERINE DRY WEIGHT
Ovariectomized controls without any treatment	25	115.5 ±3.3	22.4 ±0.65
Ovariectomized 0.1 μg Estradiol 17B	36	227.3 ± 6.1	42.3 ±1.8
Ovariectomized 0.1 μg Estradiol 17B + 0.5 mg ACTH	10	156.9 ±7.9	29.2 ±1.3
Ovariectomized and Adrenalectomized 0.1 ug Estradiol 17B	7	249.7 ±13.2	45.1 ±2.4
Ovariectomized and Adrenalectomized 0.1 μg Estradiol 17B + 0.5 mg ACTH	10	251.7 ±13.5	47.0 ±2.3

This table demonstrates that the inhibition by corticotropin of estradiol-induced uterine growth is mediated by the adrenal glands.

DISCUSSION

The therapeutic problem of dealing with certain endocrinopathies is complicated by the recognition that a target organ probably does not respond simply to one hormone alone, but more likely to the total internal hormonal environment of the patient. Thus, in both frank myxedema and in hyperthyroidism there is consistently observed a marked dysfunction of the female reproductive system, manifested by either amenorrhea or menorrhagia. It is not yet clear, however, whether thyroid disease causes this abnormality by interfering with the release of gonadotropins from the anterior pituitary, by altering ovarian function, or by a direct effect on the uterine mucosa itself (8).

In recent years evidence has been accumulating that the so-called Stein-Leventhal syndrome of polycystic ovaries is another clinical endocrinopathy that may in a similar fashion involve more than one system of glands of internal secretion. The direct surgical approach on the ovaries

recommended by earlier workers (9) first suggested that this disease was limited to the ovaries, yet wedge resection did not invariably reverse the clinical trend. Several reports have recently appeared, moreover, drawing attention to the possibility that in some cases adrenal hyperfunction may be basically involved in the hirsutism, the ovarian lesions, and the endometrial activity (10, 11). One study documents the possible role of anterior pituitary disease in the syndrome of polycystic ovaries (12). It becomes clear, then, that in this complicated picture it is well nigh impossible for the clinician to attack with assurance only one of the several possible sites of endocrine disorder that may contribute to the clinical complaints of hirsutism, amenorrhea and sterility. The only logical clinical approach would seem to be through a series of hormonal tests, the results of which may be used as a basis for a rational combination of therapeutic regimens (12).

The laboratory test animal, however, offers one clear advantage in the eventual clarification of these complicated glandular interactions. Although it may not be possible to reduplicate a characteristic clinical syndrome in an animal, yet the results of ablation of one or another of the endocrine organs may high-light the effect of certain hormones or their metabolic derivatives on an endocrine receptor-organ. In this respect, the present report may be of some significance.

By the use of ovariectomized rats under stimulation with a standard regimen of estradiol-17β, the growth response of the uterus can be anticipated. We have presented data to indicate that hydrocortisone acetate (compound F acetate) effectively diminishes this response. The halogenation in position 9 of this compound (9α-fluorohydrocortisone acetate)—a change that increases its mineralocorticoid activity—is shown to be even more effective in suppressing uterine growth. We have found other derivatives of adrenocortical hormones also to have this effect (3, 6). It is of interest that the statement has recently been made that "the exuberant uterine growth, which is induced in hypophysectomized rats by the injection of estrone" cannot be restricted by any type of steroid (13). We have not used hypophysectomized preparations in this study and it is, therefore, not possible to state categorically that the effects of mineralocorticoids on uterine growth here recorded could not have been mediated through anterior pituitary gonadotropins. On the other hand, there is no present evidence that the gonadotropins play a part in the response of the uterus to exogenous estrogen. Our data suggest that restriction of uterine growth by suprarenal steroidal compounds may be relatively specific. Thus, in our control series, pregnane 3α,20α-diol had no such effect.

Since corticotropin also inhibited uterine growth in the ovariectomized rat, it seems likely that this result may be the endogenous adrenocortical

response to anterior pituitary secretions. That this is not a direct action of corticotropin on the uterus itself, but is mediated through the adrenals, is shown by the failure of corticotropin to induce this effect in animals receiving estrogen that were both adrenalectomized and ovariectomized. This is in keeping with the report that the growth response of the uterus cannot be interfered with by any protein solution, notably those containing lactalbumin or casein (13).

In the present experiments we found confirmation of the long-established endocrine principle that the exogenous administration of a hormone suppresses the endogenous production of the same compound. This was observed in the decreased weight of the adrenal glands of those rats receiving the test solutions. Thus, administration of 1.25 mg. of compound F acetate resulted in a reduction in weight of the adrenal glands in ovariectomized control animals from 60 mg. to approximately 48 mg.

Our data furnish additional confirmation of the work of Szego and Roberts (14) and Szego (15), who showed that the uterine absorption of water caused by estradiol could be suppressed by compound F and corticotropin, using a four-hour, intravenous technique. We have found that the growth response indicated by uterine dry weights) is also inhibited.

It is exceedingly difficult to define with clarity the complex hormonal interactions that contribute to the formation of certain recognizable clinical syndromes. The laboratory offers a chance to divide certain of these complicated patterns into their component parts and thus call attention to functional pathways that may otherwise be overlooked.

The present report emphasizes in particular the possibility that adrenocortical hormones or their metabolites may have a direct influence on the ability of the uterus to respond normally to the stimulus of certain ovarian steroids.

SUMMARY

The effects of hydrocortisone, 9α-fluorohydrocortisone and corticotropin have been determined on the uterine growth induced by estradiol-17β in the ovariectomized albino rat. The results indicate that injection of as little as 0.08 mg. of hydrocortisone acetate was effective in reducing the growth-promoting action of estradiol by 37 per cent; larger doses (0.32 to 5.0 mg.) reduced this action by 40 to 50 per cent. Injection of 9α-fluorohydrocortisone acetate in doses up to 0.5 mg. resulted in a 10 to 90 per cent suppression of estrogenic stimulation of the uterus. Purified corticotropin caused a 35 to 50 per cent reduction in estradiol-induced uterine growth in the ovariectomized rat, but was without such effect in the estradiol-treated ovariectomized, adrenalectomized animals. The possible significance of such hormonal interactions in dealing with complex clinical endocrine syndromes is discussed.

Acknowledgment

We are indebted to Mrs. Betty G. Smith and Miss Nancy M. Raney for their helpful technical assistance.

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PANCREATIC ALPHA-CELL FUNCTION IN RELATION TO CHOLESTEROL METABOLISM*

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INTRODUCTION

THE function of the alpha cells of the islets of Langerhans remains an enigma despite knowledge of their existence for many years. In 1945 Thorogood and Zimmermann advanced the hypothesis that these cells produce a hyperglycemic factor antagonistic to insulin (1). During recent years many data have been obtained which appear to support this concept. A hyperglycemic-glycogenolytic factor (HGF) has been found in pancreatic extracts and has even been isolated in crystalline form. This work has been reviewed by Foa (2). However, no one has been able to prove that the alpha cells are the source of HGF. In fact, recent work negates this concept.

In 1951 Van Campenhout and Cornelis demonstrated that in the guinea pig the alpha cells of the islets of Langerhans could be selectively destroyed, by the parenteral administration of cobaltous chloride, leaving the beta cells intact (3). The cells regenerated after a period of several days. These results have since been confirmed in other species (4, 5). In experiments involving selective destruction of the alpha cells, Goldner *et al.* were unable to demonstrate that these cells produced HGF (5, 6). These workers administered cobaltous chloride to dogs and rabbits intravenously and found that the blood sugar level did not fall, as would be expected if the alpha cells were the source of HGF. They also obtained HGF from pancreatic extracts after the alpha cells had been destroyed—a further indication these cells were not the source of HGF.

This study was undertaken in order to determine whether or not the function of the alpha cells is concerned with cholesterol metabolism. It is a study of the effect of alpha-cell destruction on the level of plasma cholesterol in rabbits.

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METHODS

White male rabbits (New Zealand) weighing from 4 to 5½ pounds were used. The animals were divided into three groups, depending upon the dosage of cobaltous chloride. Varying quantities of a solution of 0.5 per cent cobaltous chloride in normal saline were injected into the marginal ear-vein. All references to cobalt pertain to cobaltous chloride.

Group I rabbits received two injections, each containing 50 mg. of cobaltous chloride, within a two-hour period. There is maximal destruction of the alpha cells with survival of the rabbit when cobaltous chloride is given twice within a two-hour period (7). Six of the animals received a similar series of 2 injections seven days after the first series (Table 1).

Group II rabbits were given a single injection of 50 mg. of cobaltous chloride. Eight days after the plasma cholesterol level had returned to normal, these animals were given two more injections (containing 50 mg. each) within a two-hour period, in the same manner as in Group I.

TABLE 1. PLASMA CHOLESTEROL LEVELS IN GROUP I RABBITS FOLLOWING TWO INTRAVENOUS INJECTIONS (EACH CONTAINING 50 MG. OF COBALTOUS CHLORIDE) WITHIN TWO HOURS

Rabbit No.	Plasma cholesterol (mg./100 ml.)															
	Days following injection															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	21
22	68	139	230	343	262			153		73		51				69	
24	51	162	226	191	193			168		69		69				57	
25	68	149	306	330	304			228		125		57				63	
26	55	130	100	65	60			84		61		51				45	
27	58	163	185	100	104			48									46
28	42	120	138	119	96			50									54
29	44	186	322	368	343			218		134							34
31	90	227	322	302	348			285		130							54
32	110		243														
33	104	168	208	153	123			61							62		
34	50	94	175	169	106			59							52		
35	99	110	151	143	90										37		
36	85	104	132	127	109			130							59		
37	78	148	198	116	96			60									
38	137	190	295	260	244			233	236		206	170			72		
39	76	126	115	116	93			52									
40	55	114	244	320													
41	57		282														
42	57	92	103	56	66			83									
43	46	194	119	76	80			61									
45	50	92	113	76	72			60									
46	51	60	77	78	67			65									
47*	47	80	98	54	73			62	100	96	66						
48*	76	114	90	58	57			52	130	118	107						
49*	62	102	104	62	58			47	190								
50	62	92	154														
51	63	126															
52	51	150	121	72													
53*	62	94	77	64	51			43	48	62							
54*	47	92	77	56	45			37	170	200							
55*	45	92	73	52				47	102	84	76						
56	69	96	60														

* The animal received an additional series of two injections on the seventh day after the first series.

Group III rabbits received a single injection of 50 mg. of cobaltous chloride per Kg. of body weight. This was the only group that received cobaltous chloride on a body-weight basis. Four of the animals of Group III received a second single injection of 50 mg. per Kg. one month after the first injection.

Blood for determination of plasma cholesterol was collected from the marginal ear-vein in oxalated tubes. The blood was spun in a centrifuge and the plasma drawn off. The plasma total cholesterol concentration was determined by the method of Pearson *et al.* (8). In about half the animals control values were obtained on three successive days, but in the remainder a single control value sufficed, for the plasma cholesterol level was found to be fairly constant in the same animal.

As soon as the control blood specimen was obtained, the cobaltous chloride was injected intravenously. The plasma cholesterol concentration was determined daily at the intervals shown in Tables 1–4. At the beginning of the study the animals were fasted overnight. This was discontinued when it was found that the control values were not altered by fasting.

Some of the animals were sacrificed by injecting air into the marginal ear-vein. In a few instances histologic sections were made after immediate fixation of the pancreas in Bouin's solution; the sections were stained by the method of Gomori (9). In 10 of the animals histologic sections of the liver were stained for fat with Sudan and hematoxylin.

RESULTS

The data show that in these rabbits the intravenous administration of cobaltous chloride caused a sustained hypercholesterolemia. The blood cholesterol level was elevated twenty-four hours after administration of the cobalt, and by the second or third day reached a peak value which was two to five times the control value. It then fell gradually and reached the control level by about the seventh day. In some instances the plasma cholesterol concentration remained elevated for longer periods, whereas in others the rise was minimal and the return to the base value occurred within twenty-four to forty-eight hours. The original response of 6 animals in Group 1 (Table 1, asterisks) was minimal. They received a second course of injections seven days later in order to determine whether they

TABLE 2. PLASMA CHOLESTEROL LEVELS IN GROUP II RABBITS FOLLOWING A SINGLE INTRAVENOUS DOSE OF 50 MG. OF COBALTOUS CHLORIDE. ON THE TWELFTH DAY AFTER ADMINISTRATION THE ANIMALS WERE GIVEN TWO INJECTIONS (EACH CONTAINING 50 MG. OF COBALTOUS CHLORIDE) WITHIN TWO HOURS

Rabbit No.	Plasma cholesterol (mg./100 ml.)																			
	Days following injection																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
17	100	182	89			70		50		56			52	122	118	166				
18	55	199	106			63		54		67			78	94	80	66	47			38
19	64	160	95			70		68		58			68	134	172	111	80			110
20	79	147	127			68		84		88			74	128	218	160	106			61
21	60	97	80			76		60					60	122	118	75	88			49

were relatively refractory or whether additional cobalt could elicit a greater response. All but 1 responded with a hypercholesterolemia which was greater than that observed during the first series of injections.

The hypercholesterolemia was not as great nor as sustained in Group II rabbits which received the smallest dose of cobalt chloride (Table 2). The peak of the rise occurred in twenty-four hours and the return to normal in about forty-eight hours. Eight days later when these animals were given a series of injections in the same manner and in the same dosage as in Group I, the results were similar to those in Group I. It is interesting that Rabbit #18 showed a rise in response to the single dose, but none when given the double dose.

The dosage of 50 mg. of cobaltous chloride per Kg. of body weight given

TABLE 3. PLASMA CHOLESTEROL LEVELS IN GROUP III RABBITS FOLLOWING A SINGLE INTRAVENOUS ADMINISTRATION OF 50 MG. OF COBALTOUS CHLORIDE PER KG. OF BODY WEIGHT

Rabbit No.	Plasma cholesterol (mg./100 ml.)																		
	Days following injection																		
	0	1	2	3	4	5	6	7	8	9	12	13	14	15	18	20	27	29	32	44
5	78	252	420	348				278		290			79	55						
6	90	220	188	130				134	143				75	72						
7	77	128																		
9	122	350	181				134			39										
1*	90				184		86													
2*	125				133		96													
3*	74				102	122		81												
4*	116				253	368	280				212	236	213	175	138	140	140	90	93	69

* The animal had received the same dose of cobaltous chloride four weeks previously.

to the rabbits of Group III (Table 3) proved to be too toxic, as 6 of the 15 rabbits died, 5 of these dying within twenty-four hours of the injection. The rest of the animals withstood this dosage well.

The elevations in the plasma cholesterol level were of about the same magnitude and duration in Groups I and III. Rabbits #1 through #4 of Group III were given a second injection of 50 gm. per Kg. four weeks later (Table 3). The typical rise and then the fall to the base level occurred both times. The plasma cholesterol level was determined by another method during the first injection of these 4 animals; therefore the results for that course are not listed. In 3 of the animals the hypercholesterolemia persisted for about five days, and in Rabbit #4 a significant elevation was sustained for about thirty days (Table 3).

If the dosage of cobaltous chloride proved too toxic, the animals usually died within twenty-four hours. However, it was noted that in 4 animals

which died twenty-four hours or more following the injection, the blood cholesterol level was greatly elevated at the time of death.

In general, the hypercholesterolemic response to cobalt was consistent in all of the animals, regardless of the amount given. In only 3 of 45 rabbits was there no significant rise.

Table 4 shows the results of the histologic examination of the liver for fatty infiltration in 11 animals selected at random. These sections were compared to a control specimen with respect to the amount of Sudan-stained material observed. In only 1 instance (Rabbit #50) was there a significant increase in fatty infiltration. We have no explanation for this 1

TABLE 4. COMPARISON OF HISTOLOGIC DEGREES OF FAT INFILTRATION OF THE LIVER (SUDAN STAINED) IN 11 RABBITS SELECTED AT RANDOM

Rabbit No.	Plasma cholesterol (mg./100 ml.) when sacrificed	Day sacrificed	Amt. of fat in liver, histologically
57	40	Control	—
38	66	3	Normal
41	282	2	Normal
47	66	3	Normal
48	107	3	Normal
49	190	1	Normal
50	154	2	4 plus
51	126	1	Normal
52	72	3	Normal
53	62	2	Normal
54	200	2	Normal
56	60	2	Normal

case, as other animals with higher plasma cholesterol values did not show any fatty infiltration of the liver.

Histologic sections of the pancreas stained by Gomori's method were prepared in several instances. Since there were only a few such preparations no attempt was made to correlate the degree of alpha-cell destruction with hypercholesterolemia. In 1 case in which the plasma cholesterol level was 282 mg. per 100 ml., no alpha cells could be found in the pancreas.

The other plasma lipid fractions were not determined. However, it was readily evident that the level of the neutral-fat fraction was elevated twenty-four hours after administration of cobalt, as shown by the extremely lipemic (milky) plasma. The plasma began to clear in forty-eight hours, but the cholesterol level remained elevated for longer periods of time. Thus the levels of two plasma lipid fractions, cholesterol and neutral

fat, were increased following administration of cobalt. It is likely that the levels of other lipid fractions were elevated as well.

DISCUSSION

The data clearly show that the intravenous administration of cobaltous chloride with resulting destruction of the alpha cells of the islets of Langerhans induces a sustained elevation of the blood cholesterol level in rabbits. This raises the question of whether or not the mechanism of the elevation is due to the destruction of the alpha cells.

The selective destruction of the alpha cells by cobaltous chloride is a well-established fact (4, 5, 6). The beta cells remain intact and no other other tissue appears to be injured. Liver glycogen has been reported to be diminished early following administration, but no morphologic evidence of parenchymal-cell damage has been demonstrated. There is evidence of alpha-cell damage one hour after administration of cobalt, and in four hours almost no alpha cells can be found. The destruction seems to be non-inflammatory and resembles the destruction of the beta cells caused by alloxan. The alpha cells disappear by autolysis without the infiltration of leukocytes. Regeneration of alpha cells occurs in about five to six days and is complete by about the tenth day.

This sequence of events coincides well in time with the changes in plasma cholesterol concentration following the intravenous administration of cobalt. A marked rise in the cholesterol level occurs within twenty-four hours, reaches a peak between the second and third day, and slowly falls to the control value by about the seventh day. Thus the destruction and regeneration of the alpha cells parallel the rise and fall, respectively, of the plasma cholesterol level.

Such an interpretation of the data implies that the alpha cells exercise endocrine control over blood cholesterol concentration and therefore must secrete a hormone that regulates lipid metabolism. Such a concept is not new and brings to mind the work of Dragstedt *et al.* (10). They suggested that the alpha cells produced a lipotropic substance (lipocaic) which prevents fatty infiltration of the liver. This hypothesis was challenged by several workers and the matter has been relegated to the status of an unsolved problem (11).

It appears that the greater the degree of damage to the alpha cells, the more prolonged the hypercholesterolemia. According to Volk *et al.*, the degree of destruction of the alpha cells can be increased by administering a second dose of cobaltous chloride within two hours (7). Thus when some of our animals were given a single injection (Group II), the hypercholesterolemia was not sustained for as long a time as when the same animals were given two injections within two hours at a later date. It is interesting to

note the variation of response in some of the animals. This can only be attributed to individual variation and resembles that observed in animals during the production of diabetes by alloxan.

In brief, the inference that destruction of the alpha cells may induce an elevation of the plasma cholesterol level, is based on these facts: 1) cobaltous chloride selectively destroys the alpha cells, leaving other tissues intact, 2) elevation of the plasma cholesterol level and the return to normal parallel the destruction and regeneration of the alpha cells, and 3) increased destruction of the alpha cells results in a more prolonged hypercholesterolemia.

The possibility of the hypercholesterolemia being the result of a process other than alpha-cell destruction must be considered. Hepatic damage caused by the cobalt, with incidental pancreatic alpha-cell destruction, comes first to mind. This hypothesis does not appear to be valid, in view of the absence of histologic damage to the liver. Moreover, there was no fatty infiltration of that organ, as shown by the sections stained with Sudan (Table 4). Parenchymal liver damage without obstruction of the biliary tree does not result in hypercholesterolemia; if a change does occur in the blood, it is a lowering of the cholesterol level rather than elevation. This is seen in cases of acute yellow atrophy of the liver (12) and carbontetrachloride poisoning (13). Study of the blood cholesterol level in hepatitis has not revealed any consistent elevation in a large series of patients (14). Thus it hardly seems likely that in our series the sustained hypercholesterolemia (which in 1 instance persisted for thirty days) could have been due to liver injury.

The possibility of the hypercholesterolemia being caused by cobalt-induced hypothyroidism must be considered, in view of the recent report by Kriss *et al.* (15). They reported the development of hypothyroidism and hyperplasia of the thyroid gland in several persons receiving cobalt therapy for sickle-cell anemia. These patients received cobalt orally for periods varying from three to seven months. Some had elevated blood cholesterol levels.

Cobalt-induced hypothyroidism could not be the mechanism for the production of the elevated cholesterol level in our animals, in view of the data. The rapidity of the elevation and the return to a normal level serves to rule out such a mechanism. One or two injections of cobaltous chloride could not induce a hypothyroid state, with resulting elevation of plasma cholesterol concentration, within twenty-four hours. Even when the thyroid gland is removed surgically, the blood cholesterol level does not rise so quickly. The return of the cholesterol to the control value in about seven days further serves to eliminate hypothyroidism. The administration of thyroid in either people or animals is not followed by such a rapid

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lowering of the blood cholesterol level. All these observations refute hypothyroidism induced by cobalt as the mechanism for production of hypercholesterolemia in these experiments.

The function of the alpha cells has been considered to be the production of the hyperglycemic factor. Despite the isolation and crystallization of HGF, no one has yet proved that the alpha cells are the source of this material. Goldner *et al.* noted a temporary rise of the blood sugar level within one hour after the administration of cobaltous chloride. That this was not due to the sudden release of HGF by the destroyed alpha cells was proved by the repeated elevations of the blood sugar level each time cobalt was injected (4). Administration of cobalt after removal of the pancreas still resulted in transient hyperglycemia (5). Since the gastric mucosa is supposed to contain cells similar to the pancreatic alpha cells which might be a source of HGF, the upper gastro-intestinal tract was removed prior to administration of cobalt. There was a temporary hyperglycemia, indicating that neither these gastric cells nor the pancreatic alpha cells were the source of the causative factor. These workers were able to demonstrate HGF in extracts obtained from the pancreas of dogs in which the pancreatic duct had been ligated and the alpha and beta cells destroyed by cobalt and alloxan (16).

Thus the function of the alpha cells remains unknown; yet they must have a function. It has been suggested that they may be precursors of the beta cells. This hardly seems likely, in view of the fact that the secretory granules of the alpha cells differ from those of the beta cells, there is a difference in staining reaction, and each type of cell is selectively destroyed by a different substance. Alloxan destroys the beta cells leaving the alpha intact, whereas cobalt does the opposite. Granules, such as are found in both types of cells, usually indicate the presence of a secretory product. It is agreed that the granules in the beta cells represent insulin. Consequently, the granules in the alpha cells in all probability represent a secretory product differing from insulin. The differences in solubility and staining properties of both types of granules attest to their distinctiveness.

If one adopts a teleologic view, then what is the function of the alpha cells? Evidence that they produce HGF is not too sound. Lipocaic, if there is such a substance, has not been proved to originate from the alpha cells. We do not claim to have proved that the alpha cells produce a hormone which controls the blood cholesterol level. However, the data show that the blood cholesterol level rises significantly when the alpha cells are destroyed and returns to the base value when the alpha cells regenerate. The time relationship is so well correlated as to strongly suggest that such a function for these cells may be proved by further work.

SUMMARY

The intravenous administration of cobaltous chloride resulted in a marked elevation of the plasma cholesterol level and destruction of the alpha cells of the islets of Langerhans in rabbits. The elevation was from two to five fold, occurred in twenty-four hours, reached a peak value by the second or third day, and then gradually fell to the control level by about the seventh day. Increasing the degree of destruction of the alpha cells by increasing the dosage of cobaltous chloride resulted in a more sustained hypercholesterolemia. The rise and fall of the plasma cholesterol level paralleled in time sequence the destruction and regeneration, respectively, of the pancreatic alpha cells. The neutral-fat fraction of the plasma was also elevated following the administration of cobaltous chloride.

Possible explanations for the hypercholesterolemia, other than the destruction of the alpha cells, have been ruled out. The function of the alpha cells remains unknown, and proof that they produce a hyperglycemic-glycogenolytic factor is lacking. Consequently, the work reported here strongly suggests that the alpha cells may secrete a hormone regulating cholesterol metabolism.

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A STUDY OF "IDIOPATHIC" HIRSUTISM; A TRANSITIONAL ADRENAL ABNORMALITY*†

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A VARYING degree of hypertrichosis is a common clinical finding in adult females (1). When hair growth is slight there may be only a cosmetic problem; when profuse, the effect may be psychologically debilitating (2) and indicative of a deep seated endocrine abnormality. A small number of women with this condition may, upon extensive clinical examination, exhibit one of a variety of endocrine disorders arising from hyperplastic or neoplastic changes in the pituitary, adrenals or ovaries (2, 3). The majority, however, for want of evidence of specific endocrine disease, fall into a group referred to as "idiopathic" or, if the history warrants, "familial" hirsutism.

The relationship between adrenocortical activity and hirsutism is clear in patients with the adrenogenital syndrome. Attempts to implicate adrenocortical hyperactivity in the pathogenesis of idiopathic hirsutism have been made previously. Studies of urinary androgens or urinary 17-ketosteroids in crude extracts have shown the level of these substances to be low, normal, or higher than normal (4–9). Cyclic increases in the urinary ketosteroids of hirsute women have been reported and related to the ovulatory (10) or luteal (11) phases of the menstrual cycle. These cyclic changes do not occur in normal women (12). Recently, the administration of cortisone has been shown to decrease the level of urinary ketosteroids and dehydroisoandrosterone-like substances in hirsute women (13, 14), an action analogous to the effect of this hormone on these substances in the adrenogenital syndrome due to adrenal hyperplasia (15, 16). Inferential

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evidence thus suggests a relationship between adrenocortical secretions and the development of hypertrichosis.

We have studied certain aspects of steroid hormone metabolism in detail under different experimental conditions in a subject with "idiopathic" hirsutism. The results of these studies provide evidence for: 1) hyperactivity of the adrenal cortex in this disorder; 2) a "transitional" syndrome, removed from normal, but different in degree and kind from the adrenogenital syndrome; and 3) the nature of the defect in this type of "idiopathic" hirsutism. It seems reasonable to suggest that the wider application of similar techniques of study would result in greater diagnostic precision and a rational therapy for this relatively common disorder.

STUDIES AND RESULTS

Hirsute Subject G24

This patient was a 24-year-old white woman seen in the Endocrine Clinic of Memorial Hospital, with the complaint of hirsutism of eight years' duration. She had first noticed excessive growth of hair on her face and chest, beginning approximately at the age of 16 years. Hypertrichosis increased steadily until, at the time of admission, there was a profuse growth of coarse black hair covering her chin, abdomen and chest. Facial hair growth necessitated shaving once daily. Pubic hair distribution had become masculine in character. Menses, which had commenced at the age of 12 years, remained regular and were unchanged in character or duration. Since the menarche she had been subject to recurrent attacks of premenstrual acne.

On physical examination, vital signs were unremarkable, weight was 138½ pounds and height was 64½ inches. The character and distribution of body tissue was distinctly feminine. Facial scarring from acne was evident. Breast development was normal. There was hypertrichosis of the type and distribution noted in the history. There were no palpable intra-abdominal masses. On pelvic examination the clitoris appeared slightly enlarged. Vaginal and urethral orifices were anatomically and functionally normal. The size of the uterus was normal to palpation. No ovarian masses were evident.

The patient was explored surgically and the internal genitalia and adrenals were found to be normal to visual and palpatory examination; adrenal biopsy demonstrated only normal tissue.

Plan for study of urinary steroids

Urinary steroids were studied during the following series of experimental periods:

1. A 9-day control period preceding the administration of any hormones. This was followed immediately by

2. Three consecutive 3-day periods during which the patient received intramuscularly 10,000 units per day of chorionic gonadotropin (A.P.L.[1]). Urinary steroids were isolated only during the second and third of these consecutive periods. Two successive 3-day control periods followed immediately.

3. Eight months later a study was made during a 5-day period when the patient

[1] A.P.L. (Ayerst, McKenna & Harrison, Ltd.), furnished through the courtesy of Dr. J. B. Jewell.

received orally 50 mg. per day of cortisone acetate. This treatment was continued for twenty-one more days. After cessation of cortisone administration for five days there were

4. Three consecutive 3-day periods during which she received intramuscularly 100 mg. per day of corticotropin (Armour H. P. ACTH) in four divided doses.

5. The post-corticotropin control period of three days (each day studied separately) followed immediately after the cessation of corticotropin treatment. Cortisone acetate was then given orally for five months; and two months after cessation of this treatment another control collection was obtained.

During the corticotropin and gonadotropin periods the patient lived on a metabolic ward and complete balance studies were conducted.

Normal women

Two normal women of approximately the same age as Subject G24 volunteered to serve as controls for the study of this patient. The results with these 2 subjects have been described in detail elsewhere (17). The only difference in treatment of the normal women and the hirsute subject was the use of corticotropin gel (Armour, ACTHAR gel) in a single daily intramuscular injection of 60 units. For the purpose of simplified presentation, only the average values for the individual compounds in the 2 normal subjects during the control and each of the corticotropin periods are listed. Although the data of these studies were not strictly comparable, in that one woman was studied over nine days whereas the other was studied for only seven days, there was no serious discrepancy introduced by averaging the values.

Chemical methods

Method E, described in another report (17), was used for hydrolysis of the steroid conjugates.[2] In brief, Method E in major details resembles Method D, described previously (18). The further fractionation of the extracts has been described (19). The other details of isolation and quantitative analysis were as reported in the preceding communications.

RESULTS

The quantitative values for those 17-ketosteroids isolated and measured are shown graphically in Figures 1–3 and in Table 1. These compounds[3] included: 1) the 11-oxygenated derivatives, 11β-hydroxyandrosterone, 11β-hydroxyetiocholanolone and 11-ketoetiocholanolone, which reflect the end-results of all the processes of synthesis, metabolism and excretion of hydrocortisone and related adrenal hormones; 2) the 11-desoxy metabolites, androsterone and etiocholanolone, which reflect the

[2] The enzyme, β-glucuronidase, used for hydrolysis was obtained from the Warner-Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Co., New York; it is now commercially available under the trade name Ketodase.

[3] In chemical terminology these compounds are named as follows: 11-ketoetiocholanolone is 3α-hydroxyetiocholane-11,17-dione; 11β-hydroxyetiocholanolone is 3α,11β-dihydroxyetiocholane-17-one; 11β-hydroxyandrosterone is 3α,11β-dihydroxyandrostane-17-one; etiocholanolone is 3α-hydroxyetiocholane-17-one; and androsterone is 3α-hydroxyandrostane-17-one.

TABLE 1. QUANTITATIVE VALUES FOR URINARY KETOSTEROIDS IN A HIRSUTE FEMALE DURING CONTROL AND THERAPY PERIODS*

COMPOUNDS	URINARY KETOSTEROIDS (mg./24 hrs.)												
Total C_{19}-11-oxysteroids	2.9	2.5	2.7	3.2	2.5	2.3	3.4	6.8	11.1	3.6	1.9	2.3	2.0
Total C_{19}-11-desoxysteroids	14.2	14.9	12.2	16.7	13.3	2.3	17.5	18.1	20.4	9.4	4.4	4.7	6.7
3α-Hydroxyeticholane-11, 17-dione	0.8	0.9	0.7	1.1	0.8	1.2	1.1	1.8	2.6	1.1	0.5	0.5	0.6
3α,11β-Dihydroxyeticholane-17-one	1.1	0.8	0.6	1.0	1.0	0.9	0.7	2.4	4.1	1.7	0.9	0.8	0.4
3α,11β-Dihydroxyandrostane-17-one	1.0	0.8	1.4	1.1	0.7	0.2	1.6	2.6	4.4	0.8	0.5	1.0	1.0
Etiocholanolone	9.2	8.7	7.5	10.3	8.2	1.5	10.8	11.9	14.3	6.6	3.1	3.0	3.5
Androsterone	5.0	6.2	4.7	6.4	5.1	0.8	6.7	6.2	6.1	2.8	1.3	1.7	3.2
β Ketonic KS	0.6	0.5	0.6	0.4	0.4	0.1	0.6	0.6	0.3	—‡	—‡	—‡	0.3
α Ketonic KS	22.2	27.6	23.6	28.5	21.3†	6.9	28.2	34.4	40.1	—‡	—‡	—‡	10.8
Ketonic KS	25.1	27.9	25.5	29.3	24.3	7.8	28.8	33.3	39.8	17.6	9.9	10.6	12.6
Crude KS	27.0	31.1	29.2	30.0	28.6	12.6	37.2	37.5	43.6	24.7	17.4	18.9	18.7
No. of days in collection period	*9*	*3*	*3*	*3*	*3*	*5*	*3*	*3*	*3*	*3*	*1*	*1*	*10*
Period No.	*1*	*3*	*4*	*5*	*6*	*7*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
Therapy	*Control*	*Chorionic gonadotropin*		*Control*		*Cortisone acetate (E), 50/mg./d orally*	*Corticotropin (ACTH), 100 mg./d, i.m.*			*Post-corticotropin*			*Control*

* Androstane-3,17-dione, etiocholane-3,17-dione and 3α,17α-dihydroxypregnane-20-one were present in the α-ketonic fraction of all the periods examined. These substances were measured quantitatively by the Zimmermann reaction but values were not included in the table because the two diones were incompletely separated and the color correction factor for 3α,17α-dihydroxypregnane-20-one is so large that slight errors are greatly magnified. In general, however, the amount of these substances was proportional to that of the 11-desoxysteroids. The nonketonic fractions during the control and corticotropin (ACTH) periods were chromatographed and found to contain the following compounds: Δ^{16}-androstene-3α-ol, pregnane-3α,20α-diol, etiocholane-3α,17β-diol, etiocholane-3α,17α-diol, pregnane-3α,17α,20α-triol, cortolone, β-cortolone, cortol, β-cortol and 3α,11β,17α,21-tetrahydroxypregnane-20-one together with four other as yet unidentified compounds, probably steroids. No attempt was made to obtain quantitative values for these compounds but the amount of any substance identified was not grossly increased as is, for example, pregnane-3α,17α,20-triol in the true adrenogenital syndrome. The separation of these nonketonic components was incomplete and probably other steroid hormone metabolites were present in mixtures or in trace amounts.

† Value only for enzyme hydrolyzed fraction.

‡ Not separated into α and β fractions.

corresponding end-results for the actual or potential "androgen." In Figure 1, comparative steroid values are shown for hirsute Subject G24, during the control period, and for the normal subjects during the control and the subsequent corticotropin periods. The significantly increased secretion of all steroid metabolites by Subject G24 established the existence of adrenocortical hyperactivity. Within the confines of this general increase, however, there was a disproportion between the relative levels of 11-oxygenated and 11-desoxy steroids. The amount of 11-oxygenated steroids excreted by Subject G24 was at a level of adrenal activity higher than normal, but clearly less than she could produce under exogenous corticotropin stimulation (Fig. 2). Likewise her control level of 11-oxygenated ketosteroids was lower than could be produced in the normal subjects by the administration of corticotropin (Fig. 1). The excretion of 11-desoxy metabolites in Subject G24 during the control period, on the

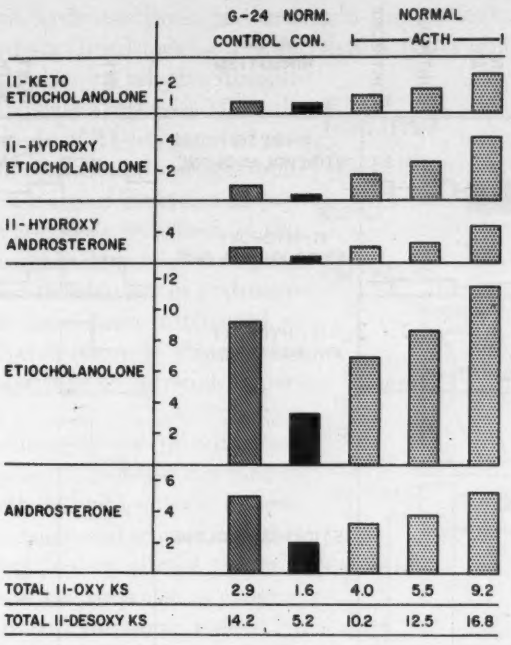

FIGURE 1

other hand, was considerably in excess of that observed in the normal women and was comparable with the excretion in the normal women when intensively stimulated by corticotropin. Figure 2 includes a comparison of the steroid excretion of Subject G24 during the control period and during the 5-day period of cortisone administration. With the exception of metabolites of the administered cortisone, there was a prompt and striking decrease in the ketosteroids measured—especially marked with respect to androsterone and etiocholanolone. Figure 2 likewise shows the steroidal metabolites during the continued administration of chorionic gonadotropin. There were no significant changes in the level of any hormonal metabolites studied. A detailed balance study conducted during this period yielded no evidence of significant metabolic changes. The quantitative data on steroid isolation during the 9-day period of corticotropin administration (Fig. 2) show that the 11-desoxy steroids increased slightly, solely as a result of higher production of etiocholanolone. There was, nonetheless, *a progressive and marked increase in the excretion of 11-oxygenated steroids*—an unmistakable reflection of increased adrenal secretory activity. This clear demonstration of a pronounced adrenocortical response, as defined in chemical terms, did not manifest itself in significant changes

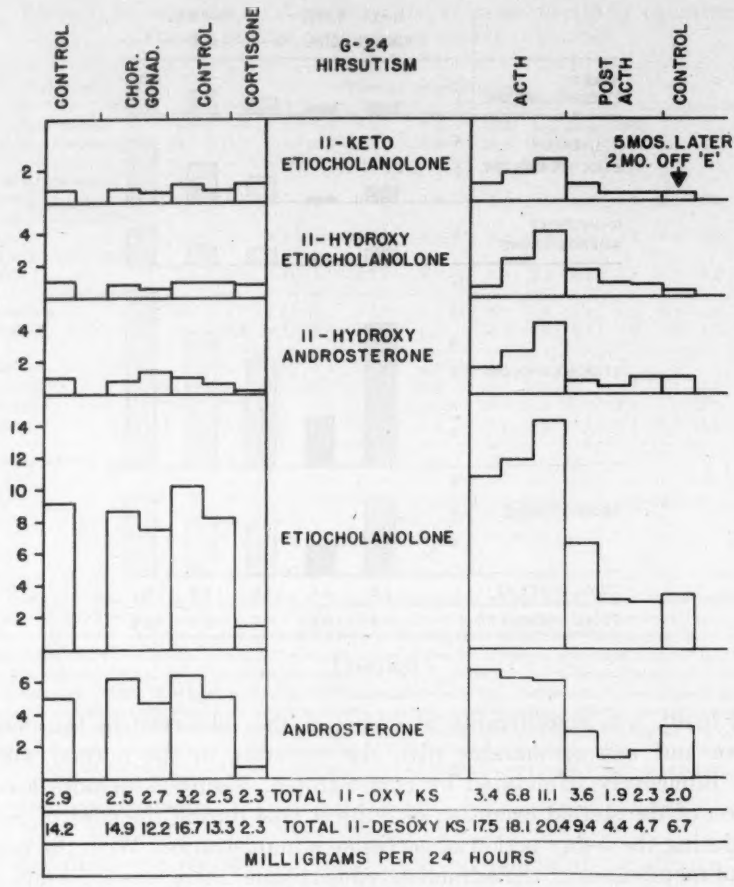

FIGURE 2

in the balance of nitrogen, phosphorus and calcium (Fig. 3). There was, however, an initial positive balance of sodium and chloride, as well as a large but transitory potassium diuresis (not shown).

DISCUSSION

The evidence for the coexistence of adrenocortical hyperactivity and hypertrichosis in Subject G24 is unequivocal. The increased levels of both 11-oxygenated and 10-desoxy steroid metabolites, together with the physiologic manifestation of hormonal excess implied in the hirsutism, constitute well defined evidence of heightened glandular secretion. There were no

signs of deficient hydrocortisone production in this patient. Her capacity for vigorous and sustained mental and physical activity contradicted any suggestion of absolute or relative diminution in hydrocortisone synthesis. More objectively, the levels of 11-oxygenated steroids during the control and corticotropin periods were evidence of active, and perhaps more than adequate, synthesis of this hormone. The establishment of these facts is pertinent, since deficiencies in hydrocortisone synthesis have been implicated as the primary adrenal lesion in the adrenogenital syndrome due to adrenal hyperplasia (20).

. The clinical evidence offers no indication that the ovaries were overtly concerned in any manner with the physiologic expression of excessive hormonal secretion in this patient. The chemical nature of the hormonal output and the response to exogenous corticotropin and cortisone, judged by the hormonal metabolites, underscores the essential role of the adrenal cortex in this disorder. The failure to elicit chemical or metabolic evidence of significant hormonal activity following vigorous treatment with chorionic gonadotropin emphasizes this conclusion. It is obligatory, therefore, to consider in detail the features of the adrenocortical secretion of this patient.

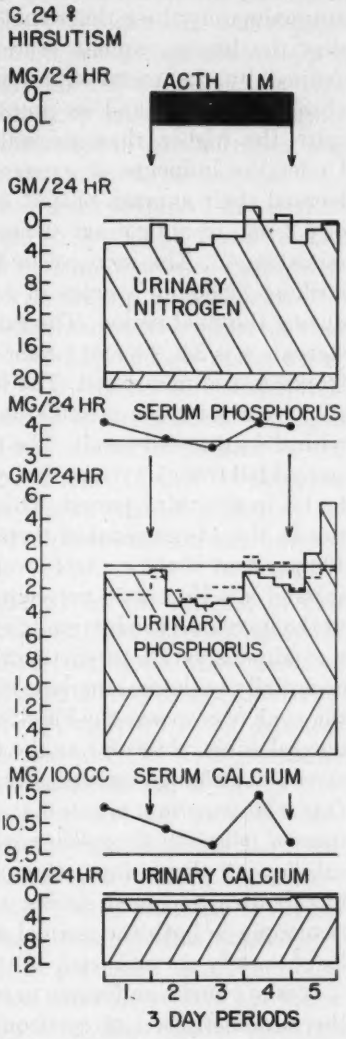

FIGURE 3

Comparison of the steroidal excretion of the normal subjects with that of the hirsute subject reveals major differences in the relative proportions of 11-desoxy and 11-oxygenated steroids produced under control conditions. The differences can be examined from the ratio of the sum of the 11-desoxy steroids to the sum of the 11-oxygenated metabolites. The average value of this ratio for the 2 normal subjects was 3.3, and both these women had essentially identical amounts of each class of metabolites. For the hirsute subject this ratio was 5.1, calculated from the average ex-

cretion during the first three control periods (Nos. 1, 5 & 6). Production of 11-oxygenated metabolites by the hirsute subject was approximately twice that of the normal subjects, but production of the 11-desoxy ketosteroids was approximately three times that of the normal subjects. It is evident, then, that the hirsute woman secreted an excessive amount of androgen, as defined by the metabolites androsterone and etiocholanolone—both in absolute amount and in proportion to the 11-oxygenated steroids—despite the higher than normal level of the 11-oxygenated compounds. Under the influence of exogenous corticotropin, the normal females increased their average output of 11-desoxy steroids from the control level of 5.2 mg. to an average value of 16.7 mg. during the final period of administration. The increase in 11-oxygenated metabolites was even more striking—from an average of 1.6 mg. during the control period to 9.2 mg. during the final period. The ratio of 11-desoxy steroids to 11-oxygenated steroids was 2.6, 2.3 and 1.8 for the three consecutive periods when corticotropin was administered. The hirsute subject responded to intensive exogenous corticotropin stimulation by a different steroidal pattern, though with the same end-result. The ratio of 11-desoxy steroid to 11-oxygenated steroid fell from 5.1 in the first corticotropin period to 2.7 in the second and to 1.8 in the third period. This was achieved, however, by a pronounced rise in the 11-oxygenated metabolites, which increased to approximately 400 per cent of the control level. On the other hand the 11-desoxy steroids showed less than a 50 per cent increase over the control level, due solely to the increased production of etiocholanolone. It would appear, then, that the administration of corticotropin to the hirsute woman resulted in an essentially unilateral increase of the 11-oxygenated steroids, so that during the peak of response the hirsute subject resembled the similarly stimulated normal subjects insofar as the adrenal secretions were concerned, with an elevated, although intrinsically "normal," synthetic and secretory pattern. *It is most important to note that this normal pattern persisted in the immediate interval following the withdrawal of corticotropin* (Fig. 2). Thus, during the subsequent three days, the proportion of 11-desoxy to 11-oxygenated metabolites remained similar to that seen during administration of corticotropin in both the normal and the hirsute subjects, *although at a much lower, nearly normal, level.*

It is of equal significance to consider the urinary steroid response during the administration of cortisone acetate. In this period, the metabolites of endogenous adrenal hormones were at significantly lower levels than during the control period (excluding 11-ketoetiocholanolone and 11-hydroxyetiocholanolone, which are metabolites of exogenous cortisone). The proportion of 11-desoxy and 11-oxygenated steroids, however, was again altered in the direction of the ratio observed in the normal subjects.

Whatever the mechanism for this prompt response to cortisone, so far as the criteria applied, adrenocortical synthetic and secretory activity was "normal" (*i.e.*, proportionate) during this period. Thus, the hormonal situation of the patient, judged by the urinary ketosteroids, was characterized by an elevated, disproportionate steroid production when she was untreated, by a proportionate and seemingly normal secretion at a very high level during corticotropin administration, by an essentially normal secretory pattern immediately after discontinuance of corticotropin, and by a low-normal level during oral administration of cortisone acetate.

It is significant that all the changes in ketosteroid output of this patient during the several treatments were confined to the alpha fraction. The beta-ketosteroids were at a normal level and were not increased significantly even during the height of response to corticotropin. Since dehydroisoandrosterone is the chief component of the beta fraction, it is apparent that the production of this compound was not materially altered under basal, stimulated or depressed adrenal function. It is, therefore, improbable that dehydroisoandrosterone served as the precursor of the adrenal androgen or was itself the cause of the androgenic manifestations implied in the hirsutism. The low level of dehydroisoandrosterone, further, is evidence against any neoplastic alteration, since it is known that an increased excretion of this compound is associated with many tumors of the adrenal gland.

The diversity of secretory activity displayed by the adrenal of this patient was remarkable. A primarily unidirectional hyperactivity of androgen secretion was evident under basal conditions. The sensitive response to cortisone administration established that this hyperactivity was not due to a neoplasm. The hyperactivity, moreover, was manifest without chemically evident alteration in the pathways of hydrocortisone biosynthesis, as well as without any clinical manifestation of hydrocortisone deficiency. Finally, this unilateral hypersecretion was seemingly dependent upon a critical level of adrenocortical function, since both the suppression induced by cortisone and the stimulation induced by corticotropin were sufficient to re-establish a normal secretory pattern.

The "transitional" nature of the syndrome found in this hirsute girl may be explained in the following terms. It is presumed that functional adrenocortical activity is directed into at least two independent synthetic routes—the one concerned with elaboration of hydrocortisone and the other concerned with the synthesis of an androgen, the chemical nature of which is unknown but which has a definite physiologic activity and is less androgenic than testosterone. Both synthetic pathways are controlled by corticotropin and may be stimulated by the administration of this hormone or, conversely, suppressed by exogenous cortisone. Prior to any

treatment of hirsute Subject G24, both synthetic paths were operative at a level higher than that characteristic of the normal individual and with androgen production at a higher relative level than that of hydrocortisone. The androgen secretion, although not maximal (as shown by the increase observed with corticotropin stimulation), was very nearly so, judged from the relatively small 11-desoxy steroid response compared with that found in the normal girls. The essentially unilateral rise in the production of 11-oxygenated steroids during corticotropin administration reinforces the conclusion that androgen secretion was near the maximum.

With this interpretation in mind, it seems reasonable to suggest that even in the adrenogenital syndrome a considerable portion of the androgen produced may arise from the type of adrenal hypersecretion described in this hirsute girl, rather than as a result of adrenal stimulation by a secondary means resulting from hydrocortisone deficiency. The defect of insufficient hydrocortisone production might be simply superimposed on the pre-existent adrenal aberration. In these terms, then, the difference between the two disorders is of both degree and kind, and hirsutism in association with this type of steroid excretion should be considered a separate syndrome.

The existence of a proportionate and apparently normal pattern of steroid excretion in the short intervals following the cessation of corticotropin therapy, together with the fact that after prolonged cortisone therapy the patient has not as yet reverted to her previous high level of steroid production, raises an interesting problem of interpretation. In the present instance it is impossible to decide which treatment resulted in the improved status, so far as steroid production is concerned. It is noteworthy, however, that patients with the true adrenogenital syndrome whose steroid production has been maintained at low levels by treatment with cortisone, rather promptly revert to their previous high levels of steroid production when cortisone is withdrawn. Regardless of how the effect was achieved, the fact that this patient has maintained an approximately normal steroidal excretion pattern for a long period without therapy is further indicative of the distinction between this state and the true adrenogenital syndrome, and serves to emphasize the "transitional" nature of the condition. It seems highly probable that between this syndrome and the true adrenogenital syndrome on the one hand, and the normal female status on the other, there may be an infinite variation in each direction. Preliminary investigations in these laboratories, together with reports of similar studies in hirsutism, indicate that there may be marked, moderate, or no elevation in steroid excretion coexistent with the disorder (21). Further studies of the type described in this report may clarify some of the problems raised in the discussion. The fact that an essentially normal

urinary steroidal pattern was achieved after cessation of treatment clearly merits further investigation.

Note added in proof

Since the preparation of this manuscript additional control studies of the steroid excretion pattern of the hirsute subject while she was without therapy have been completed. The first of these was obtained six months after the last control study reported in this article; the second was obtained more than seven months later, when all therapy had been withdrawn for eleven months. At this time, except for a two-month interval, she had received no cortisone for sixteen months. The amount of each steroid hormone metabolite measured in both of these post-treatment periods was almost precisely the same as that found in the last control period described in the main report. *It is thus evident that the steroid hormone production of this subject has been adjusted to a new level, well within the range found in normal subjects, and that, without intervention, this new level has been maintained for at least eleven months.* The implications of these results will be dealt with in further studies from these laboratories.

SUMMARY

The urinary ketosteroids of a young woman with "idiopathic" hirsutism have been studied during several experimental periods. The results provide clear biochemical evidence of abnormal adrenocortical function. The data moreover define in detail the nature of this abnormality. It is characterized by the selective hypersecretion of an adrenal androgen, as shown by a pronounced increase of androsterone and etiocholanolone production, the principal clinical manifestation of which is hirsutism. This hypersecretory activity is manifest without neoplastic change or associated defects of hydrocortisone synthesis. Nonetheless, the chemical characteristics of the defect and the response to exogenous hormone administration suggest what is termed a "transitional" relationship to the adrenogenital syndrome due to adrenal hyperplasia. It is considered extremely likely that there is a whole category of such cases, now classified as "idiopathic" hirsutism, in which hypertrichosis is a manifestation of a similar, subtle abnormality in adrenocortical activity. The application of these methods of analysis to the study of larger groups of patients with "idiopathic" hirsutism is suggested.

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EFFECT OF PIPEROXANE HYDROCHLORIDE (BENODAINE) ON THE HYPERTENSION ACCOMPANYING CUSHING'S SYNDROME*

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SINCE Cushing in 1932 (1) first described the syndrome that now bears his name, several hypotheses have been proposed to explain the hypertension which usually accompanies this condition. Albright (2) suggested that the elevated blood pressure might be related to a disturbance in electrolytes. Heinebecker (3) postulated that a "depression of the secretion of the neurohypophysis or its neutralization by other hormones results in a sensitization of blood vessels to epinephrine, to renin and to the desoxycorticosterone eosinophile complex." Unequivocal experimental verification of these hypotheses is lacking. Kurland and Freedberg (4) in 1951 studied the vascular responsiveness of 3 normotensive subjects without Cushing's syndrome to intravenous infusions of norepinephrine as measured by the rise in blood pressure. They found that adrenocorticotropin (ACTH) and cortisone markedly augmented the pressor response to norepinephrine. Since corticotropin and cortisone are capable of inducing a hypertensive state in normotensive subjects (5), and since patients with Cushing's syndrome are hypertensive as well as being in a hyperadrenocortical state, the hypothesis was suggested (4) that the hypertension accompanying Cushing's syndrome "may result from sensitization by increased amounts of C-11 oxysteroids to endogenously formed norepinephrine." Further evidence supporting this hypothesis may be found in the experiments of Fritz and Levine (6) on the rat mesentery. The observations of Raab *et al.* (7) with respect to desoxycorticosterone acetate offer additional support.

It has been shown (8) that the benzodioxanes lower blood pressure when it is elevated due either to pheochromocytoma or to norepinephrine infusions. Therefore, it should be possible to test the hypothesis of Kurland and Freedberg by administering one of the benzodioxanes intra-

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venously to patients with Cushing's syndrome. A hypotensive response could then be regarded as evidence in support of this hypothesis. Failure to obtain a hypotensive response to the benzodioxane infusion would indicate that the hypertension in these patients could not have been due to norepinephrine. Accordingly, we have studied the blood pressure responses of 2 hypertensive patients with Cushing's syndrome to intravenous piperoxane hydrochloride (a benzodioxane derivative[1] marketed by Merck & Company, Inc. under the trade name of Benodaine).

CASE REPORTS

Case 1

#570979. A 38-year-old white housewife was first seen at the University of Chicago Clinics in April, 1953. At that time, she complained of high blood pressure, bloating and flushing of her face and neck, a tendency to increased bruising, increased fatigue, deepening of the voice, loss of libido, increased growth of hair on the face, irregularity of the menses, dimming of vision, marked swelling of the ankles and occasional right-sided headaches of two years' duration. She dated the onset of her illness and all symptoms to 1951. At that time, during the first trimester of her fifth pregnancy, hypertension and swelling of the legs and abdomen developed. The blood pressure, which she had been told was 130 systolic, abruptly rose to 200. She suffered a spontaneous abortion of a twin pregnancy in the eighteenth week of gestation. Since that time, the patient had remained fatigued and her blood pressure was said to have remained high.

Physical examination on admission to the hospital revealed a moderately obese female whose adiposity was limited to the face and trunk; the arms and legs were quite thin. The face was round, plethoric and mildly hirsute. Blood pressure was 192/118 mm. Hg (right arm, recumbent). Funduscopic examination revealed only attenuation of the arterioles and an increased light reflex. Breasts were atrophic, with purple nipples and areolae. The heart was enlarged to percussion and there were no murmurs. Abdominal striae were present but were not purple. There were numerous ecchymoses on the legs. On pelvic examination there was nothing abnormal, except an enlarged clitoris. There was mild pedal edema. The rest of the findings were non-contributory.

Laboratory examination. Results of urinalysis, serologic test, red and white blood cell counts, and determinations of the hemoglobin level and sedimentation rate were normal. Total eosinophils by chamber count were 44 per cu. mm. The 24-hour excretion of 17-ketosteroids was 9.7 mg. The serum showed the following concentrations: sodium 141 mEq., potassium 3.3 mEq., chloride 101 mEq., and CO_2 32 mEq., per liter; pH 7.43; calcium 9.4 mg., and phosphorus 2.6 mg. per 100 ml. The level of blood urea nitrogen was 13.2 mg. per 100 ml., and of plasma proteins 7.0 Gm. per cent, with an albumin of 4.6 Gm. and a globulin of 2.4 Gm. per cent. Fifteen-minute phenolsulphonphthalein excretion was 35 per cent. The BMR was −6 per cent. The glucose tolerance curve was of a diabetic type. X-ray examination of the skull revealed a normal sella turcica. The visual fields were also normal. An intravenous pyelogram showed only a right renal stone. There was excellent function of both kidneys, which were of normal size. A roentgenogram of the chest revealed a heart that was 25 per cent oversize, but the lung fields were clear. Roentgenograms of the lumbar spine showed early osteoarthritic changes. An electrocardiogram showed left ventricular hypertrophy.

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[1] 2-(1-Piperidylmethyl)-1,4-benzodioxan hydrochloride.

At laparotomy, performed by Dr. Dwight Clark, a tumor measuring 30 × 33 × 22 mm. and weighing 14 grams was found medial to the superior pole of the left kidney in the adrenal gland. The tumor was removed. The right adrenal was found to be of normal size and consistency. Microscopic study of the stained sections by Dr. Eleanor Humphreys showed that the tumor had the appearance of a benign encapsulated functioning adrenocortical adenoma. The patient was maintained postoperatively on ACTH and cortisone for five months. The glucose tolerance curve became normal. Blood pressure fell to, and remained within, normal limits. Over a year has elapsed since operation. During this time the patient has remained asymptomatic.

Case 2

#605592. A 57-year-old Italian man was first seen at the University of Chicago Clinics in July, 1954. At that time, he complained of progressive weakness, loss of libido, and swelling of his face, abdomen and ankles for six or seven years. For the preceding fifteen months he had noticed a tendency to bruising. He had been told that his blood pressure was normal five years previously, but two years later he had been found to have hypertension. This elevation of blood pressure persisted until the date of admission.

Physical examination on admission to the hospital revealed a plethoric, short, dark-skinned man with a protuberant abdomen and thin arms and legs. There was facial and truncal obesity. His blood pressure was 200/130 mm. Hg (right arm, recumbent). There was a papulo-pustular eruption on the thighs, perineum, and lower buttocks, and several small ecchymotic areas on both forearms. The antero-posterior diameter of the chest was increased. The chest was hyper-resonant and coarse inspiratory and expiratory rhonchi were heard throughout both lung fields. The heart was of normal size and A_2 was louder than P_2; there were no murmurs. The abdomen was protuberant and showed purple striae. A normal liver edge was palpable at the costal margin and a fluid wave and shifting dullness were demonstrated. The rest of the findings were non-contributory.

The blood count and hemoglobin concentration were normal. Total eosinophils by chamber count were 44 per cu. mm. Analyses of serum showed the following values: sodium 136 mEq., potassium 4.2 mEq., chloride 103 mEq., and CO_2 32 mEq. per liter; pH 7.42. The plasma protein level was 5.1 Gm. per cent, with albumin 3.1 Gm. and globulin 2.0 Gm. per cent. The 24-hour urinary 17-ketosteroid excretion was 14 mg. The 15-minute phenolsulphonphthalein excretion was within normal limits. The glucose tolerance curve was of the diabetic type. The BMR was −7 per cent. An electrocardiogram was within normal limits and showed a left axis deviation. A roentgenogram of the chest and an intravenous pyelogram were normal. Preoperative blood pressures were in the range of 180 to 200 mm. Hg systolic, and 110 to 130 mm. diastolic.

A surgical exploration was carried out by Dr. Peter Moulder and both adrenal glands were found to be hypertrophied, the right gland weighing 14 grams. Eighty-five per cent of the left gland was removed following total right adrenalectomy. The blood pressure returned to normal levels after operation. However, since the patient is still receiving steroid replacement therapy and the blood pressure varies with the dosages employed, final evaluation of the effect of the operation on blood pressure must be deferred. Three weeks postoperatively the glucose tolerance curve was normal.

RESULTS

In these 2 patients with Cushing's syndrome, before operation, a slow intravenous infusion of 0.85 per cent sodium chloride was started and

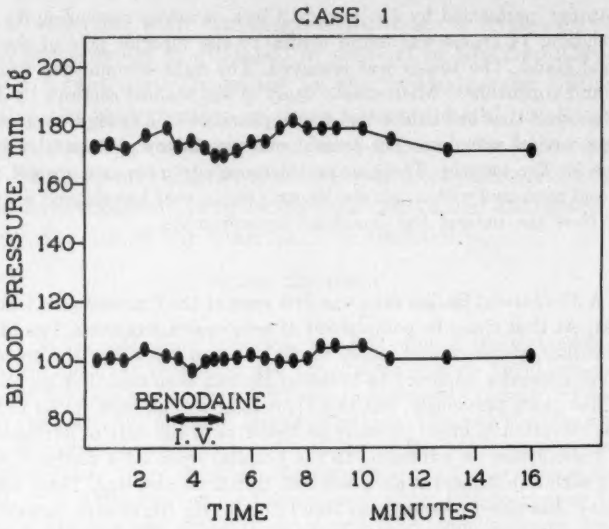

FIG. 1. Response of blood pressure to intravenous infusion of piperoxane hydrochloride (Benodaine) in Case 1. Duration of infusion is indicated by length of horizontal arrow.

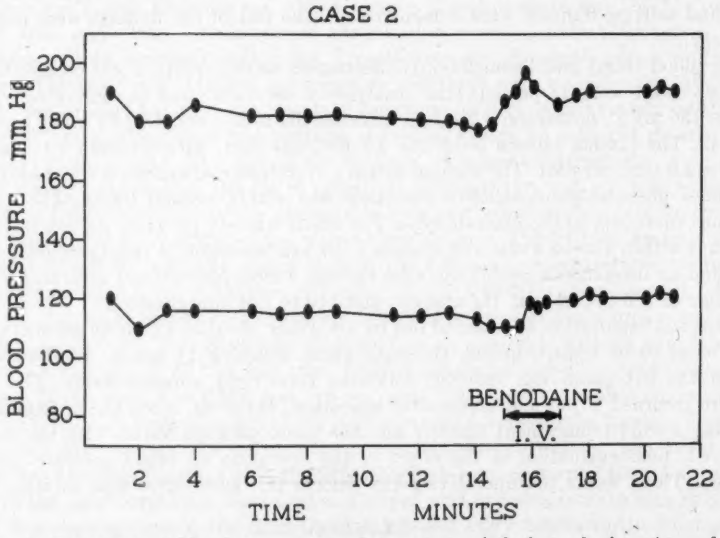

FIG. 2. Response of blood pressure to intravenous infusion of piperoxane hydrochloride (Benodaine) in Case 2. Duration of infusion is indicated by length of horizontal arrow.

sphygmomanometric blood pressure readings were recorded until a base level was obtained. Following this, piperoxane hydrochloride (Benodaine) (10 mg. per square meter of body surface) was injected into the infusion tubing and blood pressure responses were recorded. These patients at no time were given antihypertensive medication and all sedation had been discontinued for the previous twenty-four hours. The effects of piperoxane hydrochloride on the blood pressure are shown in Figures 1 and 2. Neither patient showed a significant hypotensive response to this drug.

DISCUSSION

Since piperoxane hydrochloride failed to lower blood pressure significantly in these patients, it seems justifiable to conclude that their hypertension was probably not due to circulating norepinephrine. It follows as a corollary that cortisone or other adrenocortical substances could not have raised the blood pressure in these 2 patients by means of a mechanism involving norepinephrine.[2] An alternative but improbable explanation for the lack of hypotensive response would be a possibility of excessive adrenocortical substances partially or completely obliterating the adrenolytic action of piperoxane hydrochloride on norepinephrine-induced hypertension. However, there is no evidence that such was the case.

SUMMARY

Kurland and Freedberg (4) have suggested a hypothetical pathogenesis for the hypertension accompanying Cushing's syndrome, namely, that the elevated blood pressure is due to an increased response to endogenous norepinephrine induced by excessive adrenocortical substances. This hypothesis has been tested experimentally in 2 patients with Cushing's syndrome by observing the response of the blood pressure to intravenous piperoxane hydrochloride (Benodaine). Since no depressor effect was noted, it is concluded that the hypertension of Cushing's syndrome is not due to norepinephrine and that the hypothesis under scrutiny is invalid.

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[2] The fact that the blood pressure readings in Case No. 2 are not now within the normal range (due probably to the effect of replacement therapy) does not vitiate the argument presented here, since the blood pressure of this patient was normal (or low) for over a week postoperatively. This indicates that the blood pressure was not irreversibly elevated due to generalized organic arteriolar changes secondary to prolonged hypertension.

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REFINEMENTS IN THE DETERMINATION OF "FREE PREGNANEDIOL"

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PREGNANEDIOL, as a progesterone metabolite, has been the subject of much discussion both in relation to its interpretation and to the method of its determination (1–6). Because of its application to clinical problems we have been concerned with a more precise method of assay. Pregnanediol may be measured either as sodium pregnanediol glucuronidate by the classic method of Venning (7), or as "free" pregnanediol by various modifications of the Astwood-Jones (8) method. The present paper deals with the determination of "free" pregnanediol and with the elimination of some of the steroids which may tend to give high pregnanediol values.

The use of Girard reagent is stressed as part of the routine method. Determinations were made using fluorimetry, colorimetry and gravimetry. The countercurrent distribution method was employed in an attempt to separate the pregnanediol complex in the urine of pregnant patients.

We modified and adapted the methods of Talbot *et al.* (9) and of Pincus (10) to suit our needs, since their procedures permit the quantitative determination of other steroids as well as pregnanediol in a single specimen of urine.

METHODS

The 24-hour or 48-hour specimens of urine from normal women were boiled for fifteen minutes with 10 per cent by volume of concentrated hydrochloric acid, and then extracted. Benzene was used as the solvent for one group of normal urines. The benzene extract was evaporated and the residue taken up in ether. The ether portion was separated into the phenolic and neutral fractions. The neutral fraction was then separated into the ketonic and nonketonic fractions with Girard's reagent "T."

Ether was used as the solvent for the second group of normal urines in which we employed a modified Engel (11) procedure for the separation into the phenolic and neutral fractions. The neutral fraction was further separated into the ketonic and nonketonic fractions with Girard's reagent "T."

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For each sample, regardless of the solvent used, the nonketonic neutral fraction containing the pregnanediol was evaporated to dryness and the dry residue was dissolved in 100 cc. of toluene (C.P.). The procedure which was followed for the purification and the precipitation of the pregnanediol was essentially the Talbot modification (9) of the Astwood-Jones method (8).

All precipitates were weighed prior to color development. Quantitative colorimetric determinations were made, using the yellow color developed with sulfuric acid (9), and reading with both a 420 mμ and a 520 mμ filter in an Evelyn photoelectric colorimeter. Calibration curves constructed for the pregnanediol-sulfuric acid reaction indicated that the best sensitivity range was from 0.1 mg. to 0.5 mg. of pregnanediol.

EXPERIMENTS

Steroids which may cause high colorimetric values

The possibility of the presence of digitonin-precipitable material as a source of increase in the colorimetric value in pregnanediol determination was checked on aliquots of 23 of our samples. For some of these, the method of Langstroth *et al.* (12) was used. In this method the pregnanediol itself has somewhat limited solubility and may appear in the precipitate. In several of the samples checked, a slight precipitate appeared with digitonin. Samples were then reprocessed with Girard's reagent "T," and the colorimetric determination repeated. In most cases the value remained the same on completion of the colorimetric assay. A second method (Butt *et al.* (13)), for separating digitonin-precipitable material was also used. By means of this technique we were able to detect 0.1 mg. of cholesterol and 0.5 mg. of dehydroisoandrosterone. It was also found that up to 1.25 mg. of pregnanediol would not precipitate out of solution.

It has been shown by many workers (1, 2, 3, 10) that the sulfuric-acid reaction is not specific for pregnanediol alone but that cholesterol and dehydroisoandrosterone, when present, contribute to the color. Our work confirmed these observations and showed that, weight for weight, dehydroisoandrosterone gives rise to about 66 per cent as much color, and cholesterol about 53 per cent as much color, as does pregnanediol.

The use of the Girard separation largely eliminates dehydroisoandrosterone and pregnanolone from our determinations of free pregnanediol. Cholesterol, which should form a stable colloidal suspension, filters out under most conditions (8). If contamination with dehydroisoandrosterone or cholesterol is suspected, a precipitation of an aliquot of the sample with digitonin will show it.

We also used Bongiovanni's and Clayton's method (14) for determination of pregnanediol and pregnanetriol on several of our samples prior to color development and found them to be essentially negative for pregnanetriol, which may, if present, contribute to the colorimetric value.

Smith and Barker (2) indicated that the pregnanediol values measured

as sodium pregnanediol glucuronidate are considerably higher than those for free pregnanediol and that this difference is based on what they call the non-pregnanediol components of the sodium pregnanediol glucuronidate complex. In our procedure, the use of Girard's reagent "T" eliminates this difficulty. This would be important in cases such as pregnancy, in which the 20-ketone level would be elevated. Bongiovanni and Clayton (14) also showed that the high pregnanediol values in the adrenogenital syndrome are the result of ketonic material which would be eliminated by the use of Girard's reagent "T."

The sodium pregnanediol glucuronidate complex of pregnancy

We were also interested to see if it might be possible to separate any of the sodium pregnanediol glucuronidate complex of pregnancy by countercurrent distribution. A glass Craig-Post machine with fifty tubes was used. Forty-nine transfers were made. The system was *n*-butanol-0.1 N sodium hydroxide. The aqueous (bottom) layer was extracted with butanol and this, together with the top layer, was evaporated *in vacuo*. The residues showed only one peak when each was tested with sulfuric acid for pregnanediol and for 20-ketones by the Zimmermann reaction. This indicated no separation of the complex.

Fluorimetry[1]

In addition to our colorimetric work we made simultaneous runs by the fluorimetric procedure of Linford (15), using sulfuric acid diluted to 50 per cent with alcohol. The filter system used was 435 mμ primary and 524 mμ secondary. The sensitivity to pregnanediol by this method was 8–10 gamma. The results, along with other data, are listed in Table 1. Agreement between the two methods was quite good. Considerable care has to be exercised to make the proper dilution; otherwise self-absorption presents a serious problem in fluorimetry.

RESULTS AND DISCUSSION

In Table 1 representative data have been listed showing gravimetric, colorimetric and fluorimetric results using both benzene and ether as solvents for extraction. It is important that in almost all cases the colorimetric values are lower than the gravimetric values. This is in agreement with findings of Smith (1). It is believed that the specificity of sulfuric acid for pregnanediol under the conditions applied to our analysis accounts for the difference between colorimetric and gravimetric values. It was hoped that a ratio value between the readings obtained with both 520 mμ and 420 mμ filters might give some clue as to purity of the samples when

[1] Farrand fluorimeter.

TABLE 1. PREGNANEDIOL (MG./24 HRS.)

Subject	Colorimetric	Fluorimetric	Gravimetric
T. L.	0.55	0.30	2.5
E. H.	2.60	2.60	—
E. H.	0.34	0.26	2.4
W. R.	0.90	0.64	2.0
A. K.	2.06	2.38	4.2
V. M.	10.25	10.90	14.4
M. S.	3.20	2.26	6.4
J. L.	0.70	0.60	2.4
J. S.	1.40	1.00	1.3
E. S.	0.92	1.07	2.0
E. S.	0.94	0.80	1.3
K. S.	0.64	0.44	4.4
K. S.	0.86	0.51	4.2
K. S.	1.37	1.34	5.0
K. S.	1.72	0.83	4.8
E. K.	1.32	1.52	7.2
E. K.	2.60	2.96	8.0
M. C.	0.37	0.27	1.7
M. T.	0.92	0.85	2.9
J. B.	0.37	0.21	3.2
J. B.	0.74	0.60	2.8
G. L.	1.50	1.30	5.3
P. K.	0.21	0.18	0.4
M. B.	0.80	0.53	0.8
M. B.	1.31	0.75	3.1
L. M.*	3.00	3.00	10.7
S. K.*	2.56	2.56	12.5
W. R.*	1.70	—	4.1
M. C.*	2.20	—	10.7
L. M.*	0.81	0.98	2.8
W. R.*	3.02	3.55	—

* Ether used for extraction.

the colorimetric value was compared with the weight value. Since this did not prove to be so, the data are not included.

We found it almost impossible to compare pregnanediol determinations from various laboratories, because the methods of hydrolysis and extraction and the solvents used all enter into the recovery values for 3α,20αpregnanediol. This in turn is reflected in the variety of values given for the normal range. It seems, therefore, that a group of normal values must be determined at the peak of pregnanediol excretion for each technique. It is only then that an evaluation of high or low assays can be made.

TABLE 2. BENZENE USED AS SOLVENT FOR EXTRACTION

Subject	Pregnanediol (mg./24 hrs.)	
	Colorimetric	Gravimetric
P. K.	0.94	2.9
	0.21	0.4
M. B.	0.80	0.8
	1.31	3.1
G. L.	2.47	4.7
S. K.	0.43	3.7
M. T.	0.92	1.4
M. C.	0.37	1.7
M. S.	0.17	0.8
V. P.	2.57	4.0
M. D.	0.45	1.9
	1.38	2.7
B. McV	2.20	3.8
	1.20	6.2
E. K.	0.23	0.8
	2.08	3.8
G. W.	1.39	2.6
W. R.	1.61	3.0

(Range for colorimetric values: 0.21–2.57. Average: 1.15.)

In Table 2 are listed the determinations of pregnanediol in the urines of 13 normal women. Benzene was used as the solvent. The urine specimens were collected pre-menstrually and mid-menstrually. The range of pregnanediol was from 0.2 mg. to 2.6 mg. per twenty-four hours, with an average of 1.1 mg. These values compare favorably with those in normal women as reported by Goldfine (5). The determinations in our second group of normal women, using ether as the solvent, are listed in Table 3. These values are higher than with benzene and range from 0.3 to 5.3 mg. per twenty-four hours, with an average of 2.0 mg.

CONCLUSIONS

1. The use of Girard's reagent "T" in the determination of "free" pregnanediol results in a product less contaminated by those steroids which can raise the colorimetric value.

TABLE 3. ETHER USED AS SOLVENT FOR EXTRACTION

Subject	Pregnanediol (mg./24 hrs.)	
	Colorimetric	Gravimetric
L. A.	0.62	2.3
J. S.	3.20	5.5
D. N.	0.32	1.5
J. F.	0.92	2.8
N. H.	3.40	5.7
S. R.	1.39	3.5
R. McC	5.26	8.3
B. P.	1.91	6.8
P. C.	0.79	7.9
J. K.	2.60	2.6
W. R.	0.90	2.0
	3.20	7.0

(Range for colorimetric values: 0.32–5.26. Average: 2.04.)

2. Digitonin treatment of the final sample after the use of Girard's reagent "T" did not yield digitonin-precipitable material which, when present, also increases the final value.

3. There is good agreement between the fluorimetric and the colorimetric determinations of pregnanediol with the use of Girard's reagent "T".

4. The sulfuric-acid colorimetric reaction, when applied to urine samples which have been treated with Girard's reagent "T" and purified by the Astwood-Jones method, is more specific for pregnanediol than is the gravimetric procedure.

5. Countercurrent distribution, using 50 tubes and 49 transfers with the system *n*-butanol-0.1 N NaOH, does not effect a separation of the sodium pregnanediol glucuronidate complex of pregnancy.

6. Ether extraction yields higher values for pregnanediol in normal urine than does benzene extraction.

7. Comparisons of pregnanediol values as determined in different laboratories can be made only if the methods employed are the same.

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THYROTOXICOSIS FOLLOWING ACUTE THYROIDITIS: A REPORT OF 5 CASES

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IN A recent clinical note Sheets (1) described a patient in whom hyperthyroidism followed acute thyroiditis. This coincidence was considered sufficiently unusual to warrant a case report. Barr (2) in a recent paper said, "we have found no record to indicate that in . . . thyroiditis, Graves' disease . . . has been an attributable sequel." Since it was my impression that I had seen several examples of this coincidence of syndromes, these two papers served as a stimulus for a search through clinical records of the past ten years for patients in whom thyroiditis was followed by hyperthyroidism within the course of one year.

Case 1

S.F., was a 19-year-old white male who had acute thyroiditis while he was in the U.S. Army in 1945. According to the Army records, the thyroid gland became enlarged and tender, the temperature was 101.2° F., and the pulse rate was 120 per minute. The patient, who was overweight (height, 68 inches; weight, 190 pounds) had a basal metabolic rate (BMR) of +18 per cent, a serum cholesterol level of 200 mg. per 100 ml., and a sedimentation rate of 30 mm. per hour. Three examiners corroborated the impression of acute thyroiditis.

Seventeen days after the onset of the neck discomfort the thyroiditis had begun to subside spontaneously, as shown by lessening of pain over the thyroid gland, and by a normal temperature even though the pulse rate continued at 120 per minute. The thyroid gland at this time was firm, and approximately four times normal size. The patient complained of nervousness, excessive perspiration and fatigue, and a weight loss of 11 pounds. His symptoms became increasingly severe within the subsequent three weeks and, despite a good appetite, there was a further weight loss of 5½ pounds. A diagnosis of hyperthyroidism was made.

Two weeks later the blood pressure was 140/80 mm. Hg., with a pulse rate which varied between 110 and 130. The BMR at this time was +40 per cent. The patient was prepared for surgery with 400 mg. of propylthiouracil each day, with the later addition of Lugol's solution, and thyroidectomy was performed. Histologic examination of the extirpated thyroid gland revealed marked fibrosis and extensive lymphocytic

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infiltration. The impression was thyroiditis (de Quervain's type), with areas of hyperplasia consistent with the diagnosis of hyperthyroidism.

The patient has remained euthyroid since that time, as shown by BMR's which range between −10 and +2 per cent, a 24-hour thyroidal I^{131} uptake of 27 per cent and serum cholesterol levels which range between 190 and 220 mg. per 100 ml.

Case 2

E.B., a 43-year-old Negress had a sore throat February 2, 1946. The patient was seen by her private physician, who noted some redness of the throat and a temperature of 102° with a pulse rate of 100. She was given an aspirin-phenacetin-codeine combination, and there was some temporary improvement; but three days later her neck became swollen and painful. Despite the administration of various medications, including antibiotics, directed towards the relief of the "sore throat" the discomfort in the neck subsided only slightly.

One month after the onset of this illness she was seen by the author. At this time the temperature was 98.6°, BMR +22 per cent, serum cholesterol level 180 per 100 ml., and pulse 100. The thyroid gland was approximately five times normal size. The right lobe was enlarged, firm and painless; the left lobe was also enlarged and firm, but at the extreme left border there was an area which was the site of considerable tenderness. Further questioning revealed that the tenderness had begun on the right side of the neck and had progressed towards the left side. Since there were no other symptoms or abnormal physical findings, the patient was considered to be euthyroid. No medication was given at this time.

When the patient was re-examined two months later there was manifest hyperthyroidism. Despite an excellent appetite there had been a weight loss of approximately 10 pounds. She complained of an increase in sweating and nervousness, with a tendency towards insomnia and fatigue. For the first time, a staring expression was observed although there was no frank exophthalmos. The thyroid gland was firm and was four to five times normal size. There was a tremor of the extended hands and tongue; the hands were warm and moist and the cardiac impulse was forceful. The BMR was +38 per cent and the serum cholesterol level was 160 mg. per 100 ml. The blood pressure was 150/80 with a pulse rate of 120. The patient was prepared for thyroidectomy with propylthiouracil and iodine, and operation was performed ten weeks later. Histologic examination of the thyroid gland disclosed de Quervain's thyroiditis with areas of hyperplasia and involution.

Subsequently, mild hypothyroidism developed, as shown by lethargy, fatigue, cold intolerance, a BMR of −15 per cent and a serum cholesterol level of 320 mg. per 100 ml. Desiccated thyroid was administered in a dosage of 120 mg. daily. Under this therapeutic regimen the BMR was maintained in a range between +5 and +10 per cent and the serum cholesterol between 190 and 220 mg. per 100 ml.

Case 3

A.B., a 32-year-old white woman was referred to this office in March 1952 with a diagnosis of acute thyroiditis which had responded to a course of ACTH, as suggested by Cutler (3). It recurred three weeks after cessation of this treatment. The patient complained of nervousness, weakness, tremor of the extended hands, excessive perspiration, heat intolerance, palpitation and soreness in the neck. Examination revealed a thyroid gland which was firm in consistency, extremely tender to palpation and approximately three times normal size. The temperature was 99.2° with a pulse rate be-

tween 90 and 100; the sedimentation rate was 30 mm. per hour. The 24-hour thyroidal I^{131} uptake was 42 per cent (4), serum cholesterol level 210 mg. per 100 ml. and serum protein-bound iodine (PBI) level 11.8 μg. per 100 ml. (5). The patient, however, was considered to be clinically euthyroid and she was referred back to her physician with the suggestion that she receive a second course of ACTH, together with Aureomycin orally. This advice was followed and the patient improved and appeared to be clinically well for approximately four months.

At this time she again noticed the symptoms described previously, with the exception of the soreness in the neck. In addition, there had been a weight loss of 8 pounds, despite a good appetite. The thyroid gland was enlarged to five or six times normal size and was irregular in consistency, some areas being extremely firm whereas adjacent areas felt soft and hyperplastic. The blood pressure was 160/80. The pulse was 120 to 140 per minute and bounding. There was a fine tremor of the extended hands and tongue. Lid retraction was marked and exophthalmos had developed (22 mm. left eye; 23 mm. right eye). The BMR was +80 per cent, the serum cholesterol level 150 mg. per 100 ml., the 24-hour thyroidal I^{131} uptake 84 per cent, and the serum PBI level 16 μg. per 100 ml. A diagnosis of hyperthyroidism was made. The patient refused operation and was treated with 10 millicuries of I^{131}, followed three days later by Lugol's solution in a dosage of 10 drops three times a day for four weeks. Within two months the symptoms had almost completely disappeared.

At the time of re-examinations four and twelve months later she was found to be euthyroid, both clinically and in terms of laboratory sutdies. BMR's were −5 and +3 per cent and serum cholesterol levels were 210 and 220 mg. per 100 ml., respectively.

Case 4

W.R., a 47-year-old white woman was referred with a diagnosis of acute thyroiditis. She complained of mild discomfort in the neck of ten days' duration, associated with enlargement of the neck. She reported temperatures which had been elevated sporadically to as high as 101° and symptoms of mild nervousness, increased perspiration and palpitation. There had been no significant change in her weight. On physical examination the patient did not appear to be thyrotoxic. The blood pressure was 122/74, the pulse rate was 90, and the temperature was 99.2°. The thyroid gland was approximately four times normal size and extremely firm; mild tenderness persisted in the right lobe. The BMR was −8 per cent, serum cholesterol level 230 mg. per 100 ml., 24-hour thyroidal I^{131} uptake 19 per cent, serum PBI level 8.8 μg. per 100 ml., and sedimentation rate 26 mm. per hour.

It was thought that the process was subsiding spontaneously and for this reason no therapy was recommended. The patient was seen six months later with a history of increasing nervousness, heat intolerance, palpitation and insomnia for the preceding month. A weight loss of 7 pounds had occurred, despite a good appetite. Although no exophthalmos was present (17 mm. both eyes) there was mild lid retraction and a staring expression. The blood pressure was 140/70 with a bounding pulse of 120 per minute. There was marked tremor of the extended hands and tongue, and the patient was obviously thyrotoxic. The BMR was +42 per cent, serum cholesterol level 210 mg. per 100 ml., 24-hour thyroidal I^{131} uptake 45 per cent, and serum PBI level 9.8 μg. per 100 ml. The patient was given 10 millicuries of I^{131}, followed three days later by Lugol's solution in a dosage of 10 drops three times a day for four weeks. Eight weeks later the symptoms had completely disappeared.

She has remained euthyroid to the present time. Her BMR is +10 per cent and serum cholesterol level 240 mg. per 100 ml.

Case 5

M.L., a 53-year-old white woman, was referred to the author because of nervousness, tremor, palpitation, weight loss and soreness of the neck. The thyroid gland was so tender that accurate palpation was impossible because of the amount of pain induced by even the slightest pressure. The gland was, however, obviously enlarged. The temperature was 103°, blood pressure 130/70, and pulse 120. A 24-hour thyroidal I^{131} uptake was only 7 per cent but the serum PBI level was 7.8 μg. per 100 ml. The patient was treated with 200 mg. of oral cortisone daily and three days later the symptoms had almost completely subsided. Cortisone in a daily dosage of 100 mg. was continued for five additional days.

The patient remained symptom-free for approximately eight months, and then began to notice weight loss unaccounted for by change in diet, and palpitation on ascending the stairs. Physical examination revealed a blood pressure of 130/80 and a temperature of 98.8°. The thyroid gland was firm and three to four times normal size. A 24-hour thyroidal I^{131} uptake was 35 per cent, and the serum PBI level was 7.2 μg. per 100 ml. The patient was considered to be clinically euthyroid and she was asked to return to her physician, with the suggestion that she be treated by mild sedation. Four weeks later she returned, this time manifestly hyperthyroid with a pulse rate of 120, a blood pressure of 150/80, a weight loss of 6 pounds, and marked tremor of the extended hands. The 24-hour thyroidal I^{131} uptake was 68 per cent and the PBI level was 11.8 μg. per 100 ml. The patient was given 10 millicuries of radioiodine, followed by Lugol's solution, and within the next two months the symptoms of thyrotoxicosis gradually diminished. Some palpitation on exertion persisted and there was difficulty regaining the weight that had been lost. Four weeks later, on re-examination, it was apparent that the patient had become increasingly thyrotoxic. The 24-hour thyroidal I^{131} uptake at this time was 48 per cent and the serum PBI level was 10.2 μg. per 100 ml. She was given a second dose of 7 millicuries of radioiodine. Her symptoms disappeared and she has remained euthyroid since this second dose—an interval of twenty-three months.

DISCUSSION

The occurrence of nervousness, weakness, excessive perspiration, heat intolerance, and tremor and palpitation without hyperthyroidism is not uncommon during the course of acute thyroiditis. The absence of hyperthyroidism in these patients may be demonstrated by means of the BMR, the thyroidal radioiodine uptake, and the course of events clinically. Protein-bound iodine levels may be inconsistent with the clinical picture and are usually elevated. The reason for the symptoms observed during the course of acute thyroiditis is by no means clear, but may be related to nonthyroidal hypermetabolism or possibly to the acute release of hormone from damaged thyroid tissue.

Following an episode of acute thyroiditis a patient may remain euthyroid or may become hypothyroid, depending upon the amount of destruction of the thyroid gland incident to the inflammatory process. If the remaining normal thyroid tissue is sufficient to produce the required amount of thyroid hormone, the patient will be euthyroid, but if insufficient functioning thyroid tissue is available, the patient will become hypothyroid.

Persistent hyperthyroidism following acute thyroiditis must be uncommon; yet the 5 cases described in this report, in addition to the case described by Sheets would seem to suggest a causal relationship. Whether the thyroiditis initiates some abnormal process locally in the thyroid gland which induces subsequent hyperplasia and thyrotoxicosis, or whether the brake on pituitary production of thyroid-stimulating hormone is disturbed, is idle speculation since little is known concerning the pathogenesis of thyroiditis and very little more is certain concerning the etiology of thyrotoxicosis. It is of interest that thyrotoxicosis appeared during, or immediately after the thyroiditis in one of our patients, whereas there was a delay of as long as eight months in the others.

CONCLUSIONS

Five patients are reported in whom hyperthyroidism developed immediately after, or up to eight months following episodes of acute thyroiditis. This close association of events suggests a causal relationship. Of some interest is the fact that of 2 patients who were thyroidectomized, both proved to have de Quervain's thyroiditis.

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Teaching Clinic

HERMAPHRODISM: RECOMMENDATIONS CONCERNING CASE MANAGEMENT

MANY a physician might understandably be nonplussed upon being presented for the first time with a case like the following:

> **C. L.** (HLH #B-6544), aged 6 years and 2 months, living as a boy, had been referred to the pediatric endocrine clinic of the Harriet Lane Home from another hospital where a diagnosis of female pseudohermaphrodism due to congenital adrenal hyperplasia had been established two years earlier.[1] Recommendations were sought regarding future management; the question of a reassignment of sex was raised. The parents gave a story of harrowing indecision following the child's birth. Originally given a girl's name, the baby was 6 months old when a specialist consultant unequivocally declared the child to be a cryptorchid hypospadiac male. Laparotomy was not performed at that time and the appearance of pubic hair was dismissed as unimportant. Thenceforth the child's legal status was changed to that of a male and he was given a boy's name. At the age of 6, should this child have been left to live as a boy, or reassigned to live as a girl?[2]

During the past five years, an investigation[3] of the psychology of hermaphrodism has led us to formulate several recommendations pertinent to practical clinical decisions which frequently must be made in these cases. Our experience with patients and their families has acquainted us with the confusions and perplexities they commonly face. Sometimes the services

[1] At that time a laparotomy had revealed a normal-appearing infantile uterus, fallopian tubes and ovaries. Urinary 17-ketosteroids had ranged between 21 and 22 mg. per twenty-four hours. For the next two years the child had been treated with oral cortisone, but 17-ketosteroid excretion had remained undesirably high.

[2] When seen in The Harriet Lane Home, examination revealed an adequate vagina which opened into a urogenital sinus; a normal cervix was seen on vaginoscopy. The hooded clitoris (Figure 1) measured 4.3×2.1 cm. The surgeon who saw the child expressed his opinion that an adequate penile urethra could be constructed by plastic surgery. Feminization of the external genitalia was also feasible. Psychologically this child was indisputably masculine and, since medical and surgical management of the case as a boy was eminently practicable, it was decided not to run any of the grave psychologic risks of a reassignment of sex.

[3] These studies were supported by a grant from the Josiah Macy, Jr., Foundation.

of a psychiatric specialist have been indispensable, but most of the problems proved to be such as might be adequately dealt with by the informed pediatrician, endocrinologist or other physician.

DEFINITIONS, CLASSIFICATION AND FINDINGS ON GENDER ROLE

The term, hermaphrodite, has been used by us to describe not only those individuals with completely ambiguous external genital development, but also to include all instances in which a contradiction exists between the predominant external genital appearance on the one hand, and the chromosomes, gonads, hormones or internal accessory genital structures, singly or severally, on the other hand. On the basis of these factors there have been established eight clinically recognizable varieties of hermaphrodism. Wilkins and his collaborators have recently prepared a revised classification of hermaphrodism and have discussed its occurrence in the light of current theories of embryologic sexual differentiation (1). Though the five criteria of gonads, chromosomes, hormonal sex, external genital morphology and internal accessory reproductive development serve for the diagnostic classification of any hermaphroditic individual, no one of these criteria, taken singly, is sufficient for determining the sex of rearing. For sound decisions in case management, consideration of two additional sexual variables is essential, namely, the sex of assignment in which a child has already been reared for a period of time, and the gender role and orientation he or she has established.

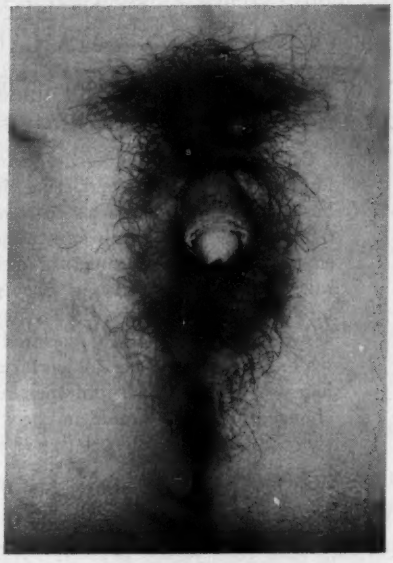

FIG. 1. External genitalia of **C. L.**; age 6 yr. 2 mo.

The gender role and orientation of an individual is a term used to indicate all those things that person says or does to disclose himself or herself as having the status of boy or man, girl or woman, respectively. It includes, but is not restricted to sexuality in the sense of eroticism. Few would dispute that the ultimate objective in the treatment of any hermaphroditic patient is to ensure the establishment and maintenance of a stable and pervasive gender role. No matter what perfection of anatomy and physiology may have been medically or surgically restored or preserved, no person

in our society could be other than crippled as an effective and happy human being without a sturdy conviction of belonging either to one sex, or the other—of being a man or of being a woman. What, then, are the factors which contribute to the establishment of a person's gender role?

The evidence from the study of hermaphroditic individuals, documented elsewhere (2, 3), can be summarized as follows: the gender role of an individual is not established at birth as instinctive, nor does it emerge in automatic fashion as though determined by the chromosomes, gonads or other somatic factors. Rather, the gender role is built up cumulatively through life experiences encountered and transacted, through casual and unplanned learning, and through explicit instruction and inculcation.

Although gender role is not an innate endowment, but rather something that is established in the course of growing up, the evidence of those hermaphrodites who have been subjected to reassignment of sex indicates that gender role, once established, is indelibly imprinted, and is not reversible or readily modifiable.

Far better than any other variable—gonadal, chromosomal, morphologic or hormonal—as a prognosticator of the gender role in an individual is the sex of assignment and rearing.

For the successful establishment of a thoroughgoing conviction of gender in the hermaphrodite it is definitely advantageous to have, from the beginning, the sex of assignment clearly and unequivocally decided, and for subsequent rearing to be consistently in accordance with that decision. Under such favorable circumstances it has, to cite one example, proved thoroughly feasible for an hermaphroditic child to grow up to psychologically healthy adulthood. Despite infertility, the presence of female chromosomes and, until surgery at puberty, normal ovaries and mullerian structures, the boy concerned grew up to be the husband in a stable and successful marriage.

Above and beyond the factors involved in establishment of gender role in hermaphrodites is the issue of the general psychologic healthiness or nonhealthiness of these individuals. Our studies (4) showed that, other things being equal, the greater obstacles to psychologic healthiness were the signs of hermaphrodism most publicly manifest, for instance hirsute virilization in women, rather than those hermaphroditic contradictions which declared themselves less publicly, as for instance genital deformities. Still less of an obstacle to psychologic healthiness were hermaphroditic discrepancies that were internal and totally concealed.

The foregoing findings in large part shape our recommendations for psychologic and clinical management. These recommendations have been set forth in detail elsewhere (5), and are herewith given only in synoptic form.

Assignment of sex: neonatal period

There are some varieties of hermaphrodism in which the external genitals at birth appear so completely masculine or feminine that ambisexuality is not suspected. Like any other baby, the infant is unhesitatingly assigned to the sex appropriate to its genital appearance, and no question is raised perhaps for years. When a baby is born with ambiguous genital appearance, it should be the aim of medical advisors to settle the assignment of sex as soon as possible. It is far preferable, however, that there be a delay of a few days, or even weeks during which investigations are made, than that there be an immediate speculative pronouncement of sex which must later be reversed. In arriving at a decision as to the assignment of sex, a major consideration should be the external genital anatomy of the neonate. If the degree of genital ambiguity is such that there appear to be reasonably promising potentials for the surgical reconstruction of serviceable and erotically sensitive genitalia, either male or female, then gonadal and hormonal considerations should be the deciding factors. The possibility of a program of hormonal intervention or control of the hermaphroditic condition, such as suppression of hyperadrenocorticism by cortisone, are cogent factors to be taken into consideration.

If the anatomy of the external genitals is so predominantly male or so predominantly female that by no possible surgical effort could they be converted to functional organs of the opposite sex, then the sex of assignment should be dictated by the external genital status, even though in some instances this would entail a gonadal or chromosomal contradiction. Subsequent medical and surgical efforts should be directed to maintaining the individual in the sex of assignment.

Reassignment of sex after the neonatal period

When hermaphrodism is not properly diagnosed until after the neonatal period, there may be a quandary of whether to change the sex already assigned in order to effect congruity between sex of rearing and the gonadal or chromosomal sex. Our recommendations in such a case are contingent on the degree to which the child has established a gender role in the sex already assigned. This is a highly individual variable, and only broad generalities may be set forth. A study of individuals who have been subjected to reassignment of sex suggests that changes may be safely recommended in the neonatal period and up to the age of around 18 months, though the parents may need help in negotiating the change. By the age of 2½ years gender role is so well established in most children that a change of sex should be considered only at the risk of serious psychologic sequelae, and with provision for psychiatric follow-up. By middle childhood, gender

role is irreversibly established and change of sex by edict should never be contemplated.

Voluntary requests for a reassignment of sex in older hermaphroditic individuals are extremely rare. When they are made, it appears that there has been, from earliest years, defective establishment of gender role. Such a request would be worthy of serious consideration if the genitals lend themselves to functional surgical adaptation in the new sex. It is doubtful, however, that, with or without a reassignment of sex, such an individual can ever establish a thoroughly pervasive and unequivocal gender role.

Surgical procedures

When, as is frequent, adaptive and restorative surgical procedures are indicated in the genital rehabilitation of hermaphrodites, questions are often raised as to the optimal timing of surgery. Our studies have shown that although it is possible for a person to establish a conviction of his or her gender despite the persistence of ambiguous or contradictory genital appearance, the doubts and perplexities arising from the anomaly constitute a handicap to the development of a thoroughly healthy personality. In infant hermaphrodites our recommendation is, in general, that surgery be as early as is consistent with surgical safety. In those instances in which surgical considerations dictate a delay in order to allow for further development of the genitals before reconstruction is undertaken, it has proved psychologically feasible for children to wait even several years, if necessary, with one important proviso. This proviso is that the child himself, as he grows up, be kept informed as to the ultimate surgical potentialities, that he understand the surgical desirability of the delay, and that in the meantime he be given some practical suggestions to help him deal with the exigencies of his peculiar genital function and appearance. For many hermaphroditic girls and boys, bathroom privacy at school should be specifically arranged for.

Partial amputation of an enlarged phallus in a girl is an operation approached with hesitation by many surgeons, in the fear that serious loss of erotic sensitivity may ensue. Studies of a limited number of hyperadrenocortical women subjected to clitoridectomy either in childhood or in adulthood (6) indicate that these women have subsequently been erotically responsive and able to experience orgasm. It has been our observation in a larger number of instances that, in girls who needed vaginal reconstruction, absence of a normal vagina is of remarkably little concern to younger girls. Such a defect becomes a psychologic issue only in early adult life, provided the patient, as a child, was not left puzzled by her anomaly. These girls are benefited by being allowed to feel free to request vaginal surgery in the teen age, if they desire it. Without such reassurance of womanly

adequacy, a girl may hold back from normal dating experiences, even though her code of behavior in any case precludes genital intimacy.

Management of parents of hermaphroditic children

Decisions as to sex of rearing and surgery are usually made by specialists. Yet the family medical advisor is perhaps the one upon whom, above all others, falls the responsibility of dealing with the quandaries and perplexities of the parents of the hermaphroditic child.

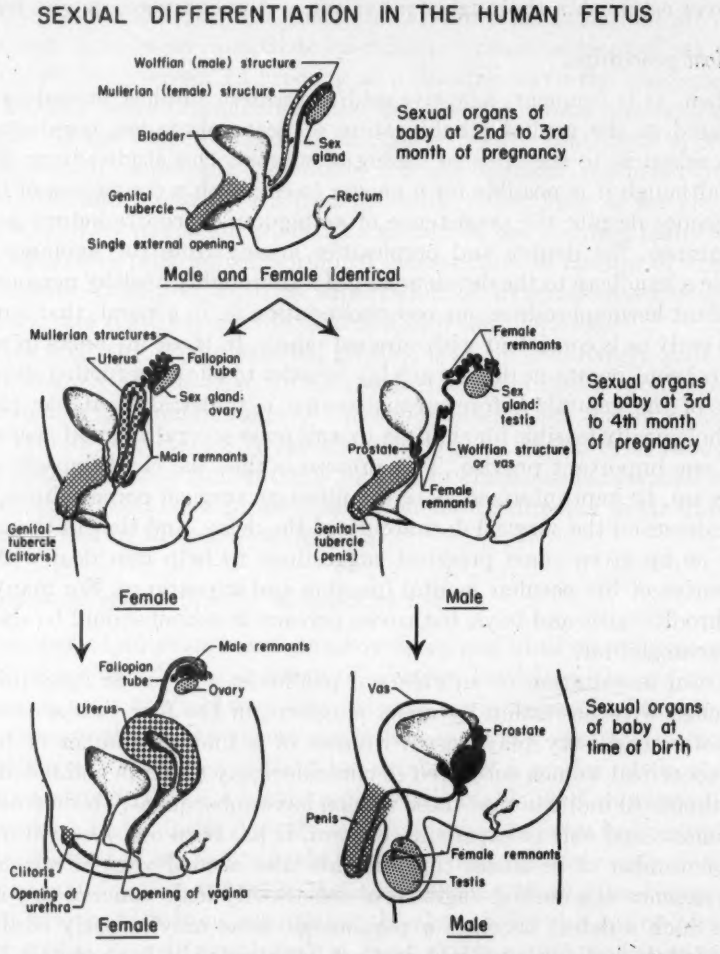

FIG. 2. Cross-section diagrams freely adpated from R. R. Greene and simplified for demonstrating to laymen the failure of complete sexual differentiation in hermaphrodism. (Reprinted from The Bulletin of The Johns Hopkins Hospital (5).)

When a genitally ambiguous infant is born, doubt as to its sex should be revealed frankly to the parents, and they should be encouraged to delay announcement of the infant's sex until an unequivocal decision is made. They should be disabused of any notion that their child is "half-and-half" or "two-sexed"—common conclusions arrived at by all but the most medically sophisticated parents. Simplified embryologic diagrams of normal prenatal sex differentiation (Figs. 2 and 3) have proved of inestimable

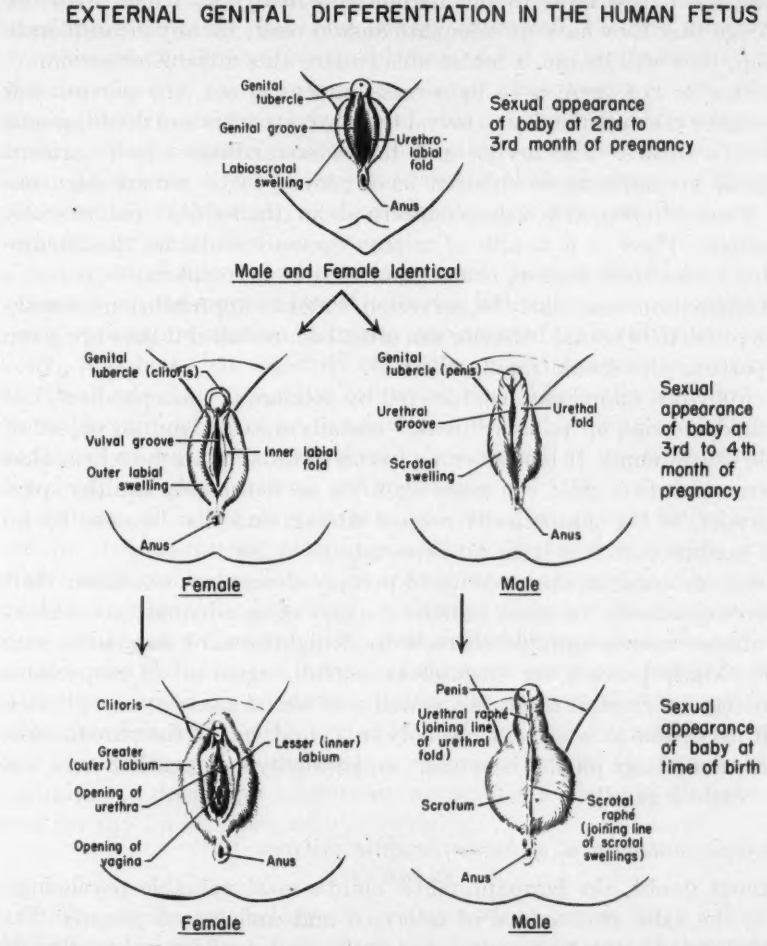

FIG. 3. Diagrams freely adapted from B. M. Patten and simplified for demonstrating to laymen the failure of complete external genital differentiation in hermaphrodism. (Reprinted from The Bulletin of The Johns Hopkins Hospital (5).)

value in relieving parental confusion, making clear to parents that their infant is, in point of fact, genitally *unfinished.*

If the sex of the infant has already been announced and a change is to be made, the parents should be given not only a thorough understanding of their infant's medical status, but practical guidance about recording the change legally on the birth certificate and other documents. They should be encouraged to talk over how they will handle the correction of announcements which have been sent out. Invariably they will need asistance in deciding what, and how, to tell friends and relatives. Armed with the knowledge that they have an adequate answer ready for any inconsiderate question, they will be much better able to face this difficult situation.

Whether or not there is to be a reassignment of sex, the parents will need to have the opportunity to reveal their private fears and doubts about the child's future. The myths and misunderstandings which torment parents of hermaphroditic children have proved to be remarkably constant. Almost universal is a deep concern about their child's future erotic inclinations. There is a wealth of misconception circulating that homosexuality stems from organic causes, and that an hermaphroditic person's sexual inclinations may likely be perverted. Parents' apprehension concerning the problem of sexual behavior can often be forestalled if they are given an opportunity to speak frankly about it.

Parents voice apprehensions, fostered by folklore of "morphodites," of their child growing up to be a "freak," socially outcast, and an object of ridicule or contempt. It is heartening beyond words for them to hear that the hermaphroditic child can grow up to be as thoroughly healthy, psychologically, as his anatomically normal sibling, and that he need by no means exclude marriage from his expectations of life.

Parents welcome an opportunity to prepare themselves to answer their children's questions. To many parents the lack of an adequate vocabulary is an almost insurmountable obstacle to straightforward discussion with a child. Many parents are themselves woefully ignorant of respectable terminology for genital structures, as well as of sexual anatomy and physiology. It may come as a surprise not only to a child, but to the parents also, to learn that a large phallus is actually an unusually developed clitoris, and not a "male organ."

Psychologic management of hermaphroditic children

Without doubt, the hermaphroditic child's most valuable psychologic asset is the calm reassurance of informed and unflustered parents. The doctor's contribution to parental certainties and confidence has already been dealt with.

One cannot conceal indefinitely from a child the fact that he or she does

not look like other boys or girls; nor should one try. The child with ambiguous genital development profits by having as early and complete an explanation of his anomaly as is within the bounds of his comprehension. Again, the concept of being genitally unfinished is extraordinarily useful in helping a child understand his condition, and in forestalling notions that he is destined to be a "freak" or "half-and-half"—misconceptions which readily emerge, even in young children, when they are given no facts or explanations of their abnormal appearance.

Perhaps even more than a normal child, the hermaphroditic child will benefit from an early and complete sex education. Biologic information, however complete, must be complemented by an explanation of moral contingencies and social proprieties. So informed, young as well as older hermaphroditic children have been able to conduct themselves with perfect appropriateness, and have been able to protect themselves from becoming objects of sexual curiosity among their playmates. Like other children, they may or may not masturbate in private, and have not been medically forbidden to do so.

As a general rule, substitutive hormonal therapy should be timed to coincide with the normal age of puberty, but individualized psychologic considerations are worthy of taking into account. If sterility can be predicted, it is best that the child be told so, with appropriate medical explanation suited to his or her comprehension. The realization that one's future parenthood must be adoptive can be a shattering blow to a late teen-ager or adult. Invariably there is less impact on one who learns this as a child, and grows up with the knowledge.

The hermaphroditic child needs to be shown, by example rather than by precept, that his private misgivings can be freely aired within the privacy of his family and to his doctor. He should come to accept that he has been unlucky enough to be born with a physical discrepancy. He may legitimately rail against the fates which thus singled him out, but, like any other handicapped child, he can learn that there are ways and means in which he can face up to his condition realistically. It is perilously easy for an hermaphroditic child, misinformed by half-truths and evasions, or frightened in the silence of no explanation, to conclude in his ignorance that his condition is a dread and mysterious misfortune, a shameful disgrace, or a cruel penalty for some legendary evil-doing.

SUMMARY

Studies of the psychology of hermaphroditic people have led to the elucidation of seven variables of sex. These variables are: 1) chromosomal sex, 2) gonadal sex, 3) hormonal sex, 4) internal accessory genital morphology, 5) external genital morphology, 6) sex of assignment and rearing, and

7) gender role and orientation—the psychosexual outlook—established while growing up. In practical decisions about assignment or reassignment of the sex of rearing, the gonadal, chromosomal and hormonal criteria, singly or together, are never sufficient. For neonatal and very young infant hermaphrodites we recommend that sex be assigned primarily, though not exclusively, on the basis of the appearance of the external genitalia and how well they lend themselves to surgical reconstruction in conformity with the sex of assignment, due allowance being made for a program of hormonal intervention, if such is indicated. For older hermaphroditic infants, children and adults, we recommend that first consideration be given to the degree that a gender role has been indelibly established in the sex previously assigned, and further, that reassignment of sex be scrupulously avoided except in rare and carefully appraised instances, in order to avoid injurious psychologic sequelae. The psychologic management of the parents of hermaphrodites and of the patients themselves derives in its specific details from a policy of frank and straightforward discussion and explanation.

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Letters to the Editor

Δ^1,9α-FLUOROHYDROCORTISONE: A NEW INVESTIGATIVE TOOL IN ADRENAL PHYSIOLOGY

To the Editor:

Until recently the results of pharamacologic studies of the absorption of hydrocortisone after topical administration in normal subjects have been difficult to interpret because it has been impossible to distinguish the absorbed steroid from that produced by the subject's own adrenal glands. Although there are ways of estimating the *total* of endogenous-plus-absorbed hydrocortisone, it is impossible to estimate each quantity individually, because we have no way of knowing to what extent the treatment resulted in suppression of endogenous hydrocortisone. The obvious solution to the problem is to employ as a subject a patient with a negligible amount of endogenous hydrocortisone, that is, one with Addison's disease or hypopituitarism. Even in this decade, however, such subjects are not always readily available.

It is now possible to carry out such studies in endocrinologically normal subjects, by virtue of the fact that Δ^1,9α-fluorohydrocortisone (Δ FF) is so effective in suppressing the secretion of corticotropin that relatively minute doses of this steroid will reduce blood and urinary 17-hydroxycorticoid levels almost to zero. Usually 0.5 mg. of Δ FF administered orally every six hours will bring 17-hydroxycorticoid values nearly to zero in less than two days. Thereafter, for as long as this suppressive dose is maintained, one may consider any appreciable rise in the 17-hydroxycorticoid level to be a result of some additional treatment, *e.g.*, the administration and absorption of either steroids or corticotropin. With endogenous 17-hydroxycorticoids "out of the way" due to the action of Δ FF, it is possible to demonstrate that considerable absorption of hydrocortisone from rectal and vaginal mucous membrane does occur, whereas absorption from the intact skin is hardly perceptible (Fig. 1). The amount absorbed can be estimated by relating the urinary values obtained on days when hydrocortisone is administered topically to values obtained on days when "standard" doses of hydrocortisone are given orally or intravenously, in the following manner:

$$\text{mg. topical steroid absorbed} = \left\{\begin{array}{c}\text{urinary corticoid} \\ \text{on day of topical} \\ \text{treatment}\end{array}\right\} \times \left\{\frac{\text{mg. standard administered}}{\begin{array}{c}\text{urinary corticoid on day} \\ \text{of standard treatment}\end{array}}\right\}$$

In a series of normal subjects, average values for absorption of topically administered hydrocortisone acetate were: from the vagina 29 per cent, rectum 26 per cent, and skin 2 per cent. Absorption in patients with Addison's disease was similar to that in normal subjects. Absorption across inflamed or abraded surfaces was not studied. It is obvious that the absorption of hydrocortisone depends upon the adequacy with which the steroid is brought into contact with the absorbing surface and that under clinical conditions this factor is difficult to standardize.

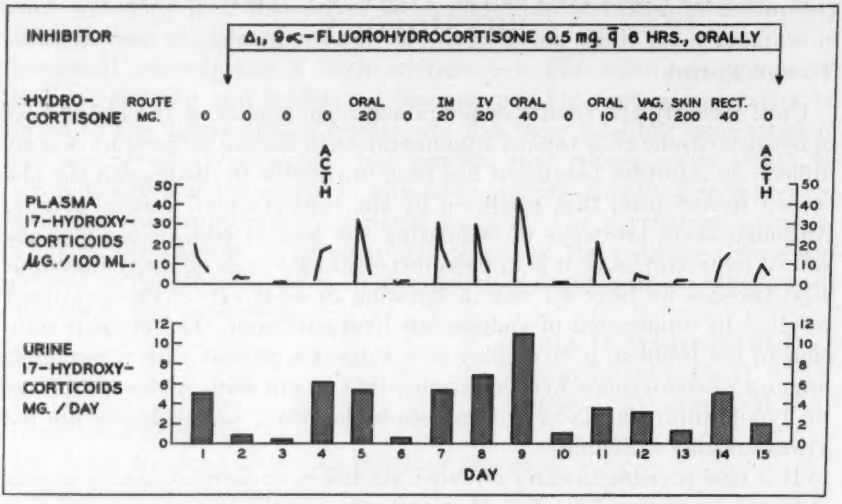
INHIBITOR
Δ1, 9α-FLUOROHYDROCORTISONE 0.5 mg. q 6 HRS., ORALLY
HYDRO-CORTISONE
ROUTE
MG.
0 0 0 0 ORAL 20 0 IM 20 IV 20 ORAL 40 0 ORAL 10 VAG. 40 SKIN 200 RECT. 40 0
ACTH
ACTH
PLASMA 17-HYDROXY-CORTICOIDS μG./100 ML.
50 40 30 20 10 0
URINE 17-HYDROXY-CORTICOIDS MG./DAY
12 10 8 6 4 2 0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAY

FIG. 1. Plasma and urinary 17-hydroxycorticoid levels in a normal subject. The methods employed were those previously described by Peterson, *et al.* for plasma (1) and by Liddle, *et al.* for urine (2). Both are modifications of the method of Silber and Porter (3). Plasma specimens were obtained at 9 a.m. (immediately before treatment), 10 a.m., 12 noon, and 4 p.m. Treatment on days 4 and 15 was with ACTH, 25 units intravenously over a four-hour period. Hydrocortisone was given orally in the form of the free alcohol, intramuscularly and intravenously as the hemisuccinate, and topically as the acetate. The topical preparations employed the following vehicles for the hydrocortisone acetate: Vaginal—lactose suppositories, lactose powder, and a cream; Rectal—suppositories and an ointment; Skin—an ointment. In the tests of transcutaneous absorption, the ointment was applied over the entire body with the exceptions of the head, hands, feet, and the areas protected by bathing suits.

The utility of Δ FF or other potent pituitary inhibitors need not be restricted to pharmacologic studies of the type described. For example, failure of Δ FF to suppress corticoid levels to near-zero may prove to be a convenient indicator of the functional autonomy of the pituitary or the

adrenal cortex, such as is encountered in Cushing's syndrome. Additional applications of the principle will doubtless occur to other investigators.

GRANT W. LIDDLE, M.D.

Section on Clinical Endocrinology,
National Heart Institute,
National Institutes of Health,
Bethesda 14, Maryland.

Acknowledgment

The author wishes to thank Dr. Avery L. Cotton of the Medical Division of The Upjohn Company, Kalamazoo, Michigan, for his generous provision of the steroid preparations used in this study.

REFERENCES

1. PETERSON, R. E.; WYNGAARDEN, J. B.; GUERRA, S. L.; BRODIE, B. B., and BUNIM, J. J.: The physiological disposition and metabolic fate of hydrocortisone in man, *J. Clin. Invest.* **34**: 1779, 1955.
2. LIDDLE, G. W.; RICHARD, J. E., and PETERSON, R. E.: An improved method for assaying the steroidogenic potency of ACTH, *Endocrinology* **57**: 594, 1955.
3. SILBER, R. H., and PORTER, C. C.: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma, *J. Biol. Chem.* **210**: 923, 1954.

RESIDUAL EXOPHTHALMOS

TO THE EDITOR:

Although the hypothesis of the thyrotropic origin of exophthalmos is based on experimental, anatomic, clinical and therapeutic data, it is not subscribed to by many authors. There have been reports of an increase of thyrotropin in body fluids in cases of malignant exophthalmos. On the other hand, there are reports to the effect that the increase, if any, is not constant. These contradictory findings are apparently due to the fact that the determinations were made at different stages in the disease. Only in the evolutional or active phase can high values for thyrotropin be found.

When the etiologic phase (excess thyrotropin) has passed, the effect (exophthalmos) can persist for anatomo-pathologic reasons (edema, infiltration, fibrosis), though the cause (hyperpituitarism) no longer exists. Thus, residual exophthalmos may be observed after remission of either euthyroid, hypothyroid or hyperthyroid exophthalmos.

Differential diagnosis. In residual exophthalmos the level of thyrotropin in the body fluids is normal or subnormal because there is no longer an excessive production of this hormone. Among the signs of excess thyrotropin, seen in the evolutional forms of the disease, are weakness, disturbances of water metabolism (leading to so-called "circumscribed thyro-

toxic myxedema" or "mucinous thyrotropic plaques"), and extrapyramidal manifestations (such as oculopalpebral abnormalities and exaggerated reflexes). In residual exophthalmos, such signs of excess thyrotropin are absent. There is no change in the exophthalmometric measurements. Because of their euthyroid state, these patients generally do not seek medical assistance except for cosmetic reasons, or because of nervousness due to anxiety about the exophthalmos.

In the author's series of cases of residual exophthalmos, 1 occurred after remission of euthyroid exophthalmos, and 3 after remission of the hyperophthalmopathic form of Graves' disease.

From the clinical point of view, therefore, it is essential to distinguish between the two forms of exophthalmos—evolutional, and residual.

Therapy. Recognition of the syndrome of residual exophthalmos is of great practical importance, because it prevents unnecessary treatment with pituitary inhibitors such as estrogens, thyroxine, hydroxypropiophenone or radiotherapy over the pituitary. Only local treatment with hydrocortisone in situ, radiotherapy over the orbits, or orbital decompression is indicated in the residual form.

From the therapeutic point of view in dealing with thyrotropic exophthalmos, the chief problem is to determine whether it is in a residual or an evolutional stage. Hypophyseal inhibitors are indicated only in the evolutional stage. In the residual form, such therapy is predestined to failure.

EMANUEL I. COHEN

Calea Călăraşi Nr. 33,
Raion T. Vladimirescu,
Bucharest, Rumania.

The Endocrine Society

THE 1956 ANNUAL MEETING

The Thirty-eighth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, Illinois, Thursday, Friday and Saturday, *June 7-8-9, 1956.*

The Committee on Local Arrangements is Dr. Norris J. Heckel as Chairman with Drs. M. Edward Davis, Sam Taylor, J. P. Greenhill, and Harley E. Cluxton as members of the Committee.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

PROGRAM

THURSDAY, JUNE 7, 1956

8:30 A.M. Registration, Foyer, Grand Ballroom, Palmer House.

I. 9:30 A.M. HYPOTHALAMUS AND PITUITARY

Chairmen: *J. W. Jailer and C. M. Szego*

1. Pituitary Adrenocortical Response to Insulin, Epinephrine, and a Non-Specific Stressor in Normal and Abnormal Patients.
   Laurance W. Kinsell, George D. Michaels, Frederick R. Brown, Jr., Roger W. Friskey* and *James E. Keasling.*
2. Physiological Studies of Adrenal Function After Hypophysectomy in Man.
   *Mortimer B. Lipsett, Charles D. West** and *Olof H. Pearson.**
3. Plasma Levels of ACTH in Normal Subjects and Addisonian Patients Following Corticosteroid Administration.
   John E. Bethune, W. Francis Ganong, David M. Hume** and *Don H. Nelson.**
4. Inhibition by Chlorpromazine of the Adrenocortical Response to Insulin Hypoglycemia.
   Nicholas P. Christy, Mary Knight, Donald Longson and *Joseph W. Jailer.**
5. Some Aspects of the Central Nervous System Regulation of ACTH Secretion.
   John W. Mason (introduced by M. S. Goldstein).* (Invited paper, 20 minutes.)

* Members.

INTERMISSION

6. Separate Hypothalamic Areas Controlling Pituitary Gonadotrophic Function and Mating Behavior in Female Cats and Rabbits.
   *Charles H. Sawyer** and *Bruce Robison*.
7. The Effect of Chloropromazine on Pituitary-Adrenal Function.
   *E. A. Ohler,** *R. W. Sevy** and *Alvin Weiner*.
8. Effect of Adrenal Hormones on the Neurosecretory Material of the Neurohypophysis.
   *Robert Gaunt,** *J. J. Chart* and *Ben Antonchak*.
9. Decreased Aldosterone Secretion in Decapitated Dogs.
   Elizabeth W. Rauschkolb and *Gordon L. Farrell* (introduced by George Sayers*).
10. Some Physiological Effects of Primate Pituitary Growth Hormone Preparations in the Hypophysectomized Rhesus Monkey.
    *Ernest Knobil,** *Richard C. Wolf* and *Roy O. Greep.**

II. 2:00 P.M. ADRENAL

Chairmen: *R. Gaunt and J. C. Laidlaw*

11. Rate of *in vitro* Transformation of Cortisone and Hydrocortisone by Dog Liver.
    *O. Hechter,** *E. Frank, E. Caspi* and *H. Frank*.
12. The Influence of Cortisol Level on the Distribution and Turnover of the Hormone.
    *Leo T. Samuels,** *Kristen Eik-Nes** and *Frank H. Tyler.**
13. The 2-Methyl-Hydrocortisones: Long-Acting Compounds of Great Sodium-Retaining Potency.
    *Grant W. Liddle,** *June E. Richard* and *Gordon M. Tomkins*.
14. Postmortem Study of Rats Made Hypertensive by Aldosterone.
    D. Kumar, W. Anderson and *A. G. Gornall.**
15. Role of Corticoids in the Production of an Experimental Polyarthritis in the Rat.
    *Hans Selye,** *Gaëtan Jasmin, Pierre Bois* and *Claude-Lise Richer*.
16. Experimental Hypertensive Vascular Disease in Rats Bearing Regenerating Adrenal Cortical Tissue: Morphologic and Pathogenetic Studies.
    *Floyd R. Skelton.** (Invited paper, 20 minutes.)

INTERMISSION

17. The Pyrogenic Effect of Etiocholanolone (3a-Hydroxyetiocholane-17-One).
    *Atallah Kappas,** *Leon Hellman, David K. Fukushima* and *T. F. Gallagher.**
18. 11β-Hydroxylase Deficiency in the Hypertensive Form of Congenital Adrenal Hyperplasia.
    *Walter R. Eberlein** and *Alfred M. Bongiovanni.**
19. Experience with a Chemical Method for the Determination of Aldosterone in Urine.
    *Luis Hernando Avandano, Don H. Nelson,** *Jean Crabbé, Eric J. Ross, Albert E. Renold** and *George W. Thorn.**
20. C^{14}-Incorporation in Urinary Steroids.
    *F. Ungar** and *R. I. Dorfman.**

21. The Half-Life of Exogenous Adrenal Corticosteroids in Health and Disease.
*Alan K. Done** and *Vincent C. Kelley.**

22. Adrenal Deficiency and Shortcomings of Cortisone Therapy in Patients with Spontaneous and Iatrogenic Adrenal Deficiency.
*A. Gorman Hills,** *Harold A. Zintel** and *David W. Parsons.**

FRIDAY, JUNE 8, 1956

8:30 A.M. Registration, Foyer, Grand Ballroom, Palmer House.

III. 9:00 A.M. REPRODUCTION AND SEX HORMONES

Chairmen: *R. Hertz and J. T. Bradbury*

23. Prolongation of Human Corpus Luteum Life Span.
*Robert A. Lyon.**

24. Genetic, Developmental and Hormonal Aspects of Gonadal Dysgenesis and Pseudohermaphrodism.
*E. Witschi,** *W. O. Nelson** and *S. J. Segal.**

25. The Sex Chromatin Pattern in Testicular Disorders: Relationship to Pathogenesis and to True Hermaphrodism.
*Melvin M. Grumbach, Earl T. Engel,** *William A. Blanc* and *Murray L. Barr.*

26. Biliary Excretion and Protein Binding of Radioactive Steroids in Human Subjects: Their Possible Relationship.
*Avery A. Sandberg** and *W. Roy Slaunwhite, Jr.*

27. Steroid Dehydrogenases as Models for the Binding of Hormonally Active Steroids.
Paul Talalay (introduced by Dwight J. Ingle*). (Invited paper, 20 minutes.)

INTERMISSION

28. Anti-Estrogen Activity of Stilbestrol Homologues at the Enzyme Level.
*Claude A. Villee.**

29. Sex Differences in Orientation of Reduction Products of 3-Keto-C_{19} Steroids by Rat Liver Homogenates.
*B. L. Rubin** and *R. I. Dorfman.**

30. Loss of Uterine Sensitivity in the Rat Following the Production of Deciduoma.
Vincent J. De Feo (introduced by Lawson Wilkins*).

31. Pituitary Independence of the Pre-Pubertal Development of the Ovary.
*Roy Hertz.**

32. Inhibition of Pituitary Gonadotrophic Secretion with Nor-Ethandrolone in Anabolic, Non-Androgenic Doses.
Jerome Goldman, Jeanne Epstein and *Herbert S. Kupperman.**

IV. 1:30 P.M. ANNUAL BUSINESS MEETING OF THE ENDOCRINE SOCIETY
Grand Ballroom, Palmer House. (All members requested to attend promptly.)

V. 2:30 P.M. THYROID

Chairman: *A. Albert and M. M. Hoffman*

33. Effect of Epinephrine and Nor-Epinephrine on the Thyroidal Release of Thyroid Hormones in Dogs.
Norman B. Ackerman and *Walter L. Arons.**

34. AN *in vitro* ASSAY FOR THYROTROPIN.
Warner H. Florsheim, Nathan Moskowitz** and *M. E. Morton* (introduced by Monte A. Greer*).
35. A NEW METHOD FOR STUDY OF IODINE METABOLISM IN THYROID SLICES.
George D. Molnar, A. Albert, F. Raymond Keating, Jr.** and *Alan L. Orvis.*
36. PLACENTAL TRANSFER OF PERCHLORATE AND TRIIODOTHYRONINE IN THE GUINEA PIG.
Sholem Postel (introduced by John B. Stanbury*).
37. IODINE METABOLISM IN SUBJECTS WITH FUNCTIONAL CARCINOMA OF THE THYROID.
J. E. Rall, J. Tata* and *J. Robbins.** (Invited paper, 20 minutes.)

INTERMISSION

38. THE SERUM PATTERN OF THYROID HORMONES IN VARIOUS STATES OF THYROID FUNCTION.
*Walter L. Arons** and *Jerrold D. Hydovitz.*
39. THE EFFECTS OF AMPHENONE ON THYROID FUNCTION IN MAN.
Herbert A. Selenkow, Alfonso Rivera* and *George W. Thorn.**
40. METABOLIC ACTIVITY *in vitro* AND *in vivo* OF THE ACETIC ACID ANALOGUES OF TRIIODOTHYRONINE AND THYROXINE.
*John G. Wiswell** and *Samuel P. Asper, Jr.**
41. MEASUREMENT OF ACTIVE IODINE STORES AND DAILY HORMONAL OUTPUT IN THE INTACT HUMAN THYROID.
John H. Nodine, Bertram J. Channick, Dimitri Sokhos, Shirley Dresner* and *William H. Perloff.**
42. NEW LABORATORY AIDS IN THE DIAGNOSIS OF HYPERPARATHYROIDISM.
E. L. Chambers, Jr., Gilbert S. Gordan,* Leon Goldman** and *E. C. Reifenstein, Jr.**

VI. ANNUAL DINNER

Warren O. Nelson, President, presiding

6:30 P.M. Cocktails, private dining room No. 14. Sponsored by the donors of the Awards of the Society: Ciba, Schering, Ayerst, and Upjohn. Attendance limited to holders of dinner tickets.

7:30 P.M. Dinner, Red Lacquer Room.
Presentation of 1956 Awards of the Society by *John E. Howard*—The Endocrine Medal, Ciba Award, Schering Scholar, Upjohn Scholar

President's Address: *Dr. Nelson*

SATURDAY, JUNE 9, 1956

8:30 A.M. Registration, Foyer, Grand Ballroom, Palmer House.

VII. 9:00 A.M. DIABETES AND METABOLISM

Chairmen: *P. H. Forsham and M. S. Goldstein*

43. THE METABOLIC EFFECTS OF PROGESTERONE IN RELATION TO THE ACTION OF ESTROGEN AND PITUITARY HORMONES.
Richard L. Landau, Delbert M. Bergenstal,* Kathleen Lugibihl* and *Dean Dimick.*
44. THE EFFECT OF SODIUM PHYTATE ON CALCIUM AND MAGNESIUM METABOLISM.
Philip H. Henneman, Evelyn L. Carroll* and *Fuller Albright.**

45. The Functional State of the Adrenal Cortex in Diabetes Mellitus.
Glenn E. Mortimore, Eleanor Irvine, James Hopper, Jr.* and *Peter H. Forsham.**
46. Differentiation of Diabetes Mellitus Due to Impaired Insulinogeneiss from that Due to Increased Insulin Destruction.
I. Arthur Mirsky, Daniel Diengott* and *Henry Dolger.*
47. Metabolic Principles of the Anterior Pituitary.
*M. S. Rabin.** (Invited paper, 20 minutes.)

INTERMISSION

48. Temporary Diabetes from Growth Hormone Alone in the Tube-Fed Rat.
J. A. Owen, Jr. and *F. L. Engel.**
49. Metabolic Effects of Testosterone in the Depancreatized Dog.
G. Costa and *C. K. Kochakian.**
50. Studies with Radioactive Glucose on the Effects of Cortisone on Carbohydrate Metabolism in Hypophysectomized Dogs.
N. Altszuler, R. Steele, J. S. Wall and *R. C. deBodo.**
51. Studies on Plasma-Insulin Activity.
J. Vallance-Owen (introduced by F. D. W. Lukens*).
52. *In vivo* Effects of Glucagon on Hepatic Glycogen and Glycogenolytic Enzymes.
George F. Cahill, Jr. and *A. Scott Earle* (introduced by James Ashmore*).
53. Tissue and Intracellular Distribution of a Glucagon Inactivating System.
H. T. Narahara, Robert W. Cox, Elaine D. Henley and *Robert H. Williams.**

VIII. 2:00 P.M. CLINICAL SESSION

Chairmen: *J. E. Howard and E. P. McCullagh*

I. Symposium on Adrenal Function, Especially as Related to Cushing's Syndrome.
A. Assay of Urinary and Blood Steroids and Responses to ACTH.
*Claude J. Migeon.**
B. Clinical Manifestations of Cushing's Syndrome; Its Diagnosis and Therapy.
*Randall G. Sprague.**
II. Diagnosis and Treatment of Diabetes Insipidus.
William C. Thomas, Jr. (by invitation).

INTERMISSION

III. Current Laboratory Methods Used in Evaluation of Thyroid Function, with Special Emphasis on Errors in Their Interpretation.
*F. Raymond Keating, Jr.** and *Sidney C. Werner.**

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.

3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE EIGHTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY; THE UNIVERSITY OF TEXAS POSTGRADUATE SCHOOL OF MEDICINE; AND THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE, HOUSTON, TEXAS

October 22-27, 1956

The faculty will consist of twenty-two eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program and registration, write to *Dr. J. B. Trunnell, M. D. Anderson Hospital, 6723 Bertner Avenue, Houston 25, Texas.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Shamrock Hilton Hotel which is adjacent to the Texas Medical Center.

The American Goiter Association

THE 1956 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in the **Drake Hotel, Chicago, Illinois, May 3, 4 and 5, 1956.**

JOHN C. MCCLINTOCK, M.D., *Secretary*,
149½ Washington Avenue,
Albany, N. Y.

PRELIMINARY PROGRAM

Thursday, May 3—8:30–12:00 a.m.

1. The Biological Activity of Pork and Beef Thyroid Preparations.
   N. R. Stasilli and *R. L. Kroc*, Morris Plains, N. J.
2. Metabolic Actions of Thyroxine Derivatives and Analogs.
   S. B. Barker, Birmingham, Alabama.
3. Additive Effects of Phosphate and Triiodothyronine in Dogs.
   K. E. Roberts, G. Firmat, P. Vanamee, J. W. Poppell and *R. W. Rawson*, New York, N. Y.
4. Effect of Estrogen on Thyroid Function.
   J. Feldman, Pittsburgh, Pa.
5. Alterations in Chick Thyroid Function Induced by Cobalt.
   J. P. Kriss, F. S. Greenspan and *W. H. Carnes*, San Francisco, California.
6. Factors Governing the Development of the Chick Embryo Thyroid. IV. The Influence of Vitamin B_{12} Deficiency on the Uptake of I^{131}, the Formation of Iodinated Compounds, and the Histologic Structure of Glands from Adult and Embryonic Chickens.
   T. M. Ferguson, J. B. Trunnell, J. R. Couch, B. Dennis and *P. Wade*, Houston, Texas
7. Perchlorate and the Metabolism of Radioiodide in Propylthiouracil-Treated Rats.
   N. S. Halmi and *R. G. Stuelke*, Iowa City, Iowa.
8. Loss of Appetite for Alcohol and Alcoholic Beverages Produced in Rats by Treatment with Thyroid Preparations.
   C. P. Richter, Baltimore, Md.

Thursday 2:00–5:30 p.m.

9. Effects of Organic Iodine Compounds on Iodine Metabolism.
   D. W. Slingerland, Boston, Mass.
10. The Diagnostic Use of New *In Vitro* Tests of Thyroid Function.
    M. W. Hamolsky and *A. S. Freedberg*, Boston, Mass.

11. Thyroid Function Studies in Children Using Radioactive Iodine.
R. M. Kohlenbrener, R. H. Kunstadter, T. Fields and L. Oliner, with the technical assistance of D. R. Goldstein, Chicago, Illinois.
12. Thyroxine-Binding by Sera of Pregnant Women.
J. T. Dowling, N. Freinkel and *S. H. Ingbar,* Boston, Mass.
13. Radiothyroxine Turnover Studies in Myxedema, Thyrotoxicosis, and Hypermetabolism without Endocrine Disease.
K. Sterling and *R. B. Chodos,* Syracuse, N. Y.
14. Metabolic Studies of Protein Metabolism in Patients with Myxedema Before and After Treatment with L-Triiodothyronine.
K. R. Crispell, G. A. Williams, W. Parson and *G. Hollifield,* Charlottesville, Virginia.
15. Comparison of Thyroid Iodine^{131} Uptakes of Treatment versus Tracer Doses.
R. Gorson, R. Chamberlain and *E. Rose,* Philadelphia, Pa.
16. Rise in Basal Metabolic Rate and Fall in Protein Bound Iodine Induced by Epinephrine.
D. R. Korst, N. S. Hiramoto and *W. H. Beierwaltes,* Ann Arbor, Mich.

Friday, May 4—8:30–12:00 a.m.

17. Van Meter Prize Essay.
18. New Laboratory Aids in the Diagnosis of Hyperparathyroidism.
E. L. Chambers, Jr., G. S. Gordan, L. Goldman and *E. C. Reifenstein, Jr.,* San Francisco, California.
19. The Physiologic Activity of Triiodo- and Tetraiodothyroacetic Acid in Human Myxedema.
J. Lerman and *R. Pitt-Rivers,* Boston, Mass.
20. An Attempt at a Physiological Classification of Cretinism.
J. B. Stanbury and *A. Querido,* Boston, Mass.
21. Inhibition of I^{131} Uptake by L-Triiodothyronine in Euthyroid Persons and Patients with Exophthalmic Goiter.
William M. McConahey and *C. A. Owen, Jr.,* Rochester, Minnesota.
22. The Significance of Low Thyroid Reserve.
William McK. Jefferies, L. W. Kelly, Jr., R. P. Levy, G. W. Cooper and *R. L. Prouty,* Cleveland, Ohio.
23. Some New Observations on Acute Thyroiditis.
Penn G. Skillern, H. E. Nelson and *G. Crile, Jr.,* Cleveland, Ohio.

Friday, May 4—2:00–5:00 p.m.

Presidential Address:

24. The Thyroidologist and Beckoning Frontiers.
Rulon Rawson, New York, N. Y.
25. Lymphosarcoma and Small-Cell Carcinoma of the Thyroid Gland.
Alexander J. Walt, L. B. Woolner and *B. M. Black,* Rochester, Minnesota.
26. Carcinoma of the Thyroid Gland. A Clinical and Pathologic Study of 300 Patients.
Stuart Lindsay, J. Friedlander, M. E. Dailey and *W. H. Rustad,* San Francisco, California.
27. Hormonal Treatment of Carcinoma of the Thyroid.
C. G. Thomas, Jr., Chapel Hill, North Carolina.

28. A Clinico-Pathological Study of 262 Surgical Cases of So-Called "Thyroiditis."
Virginia Kneeland Frantz and *A. W. Harland*, New York, N. Y.
29. Invasive Fibrous Thyroiditis (Riedel's Struma).
Lewis B. Woolner, W. M. McConahey and *O. H. Beahrs*, Rochester, Minnesota.
30. Serum "Thyroglobulin" in Hashimoto's Thyroiditis.
Charles A. Owen, Jr. and *W. M. McConahey*, Rochester, Minnesota.

Saturday, May 5—8:30–12:30 a.m.

31. Malignant Exophthalmos.
A. E. Braley, Iowa City, Iowa.
32. Levator Spasm as a Factor in Exophthalmos and Its Correction.
R. E. Moran, Washington, D. C.
33. Nodular Lesions of the Thyroid Gland in Children.
Alvin B. Hayles, R. L. J. Kennedy, L. B. Woolner and *B. M. Black*, Rochester, Minnesota.
34. Thyroid Cancer in Childhood and Adolescence.
James Majarakis, D. Slaughter and *W. H. Cole*, Chicago, Illinois.
35. The Thyroid Scintigram: An Evaluation of Its Clinical Use.
Arthur Kugel, N. Specht, R. Adams and *E. J. Boehme*, Los Angeles, California.
36. The Selection of Patients for Thyroid Surgery.
J. Martin Miller, M. A. Block, and *B. E. Brush*, Detroit, Michigan.
37. Effect of Anesthesia and Thyroid Surgery on Serum Levels of Radioactive Protein-Bound Iodine Levels.
Jerrold D. Hydovitz, Philadelphia, Pa.
38. The Complications of Thyroid Surgery.
Oliver H. Beahrs and *R. A. White*, Rochester, Minnesota.
39. A Study of One Thousand Cases of Thyroid Surgery. The Complications and Implications.
Warren H. Cole and *E. F. Fowler*, Chicago, Illinois.

YALE UNIVERSITY • GRADUATE SCHOOL
AMERICAN CANCER SOCIETY FELLOWSHIPS IN BIOMETRY AND EPIDEMIOLOGY
1956–1957

Several predoctoral and postdoctoral fellowships, supported by a grant from the American Cancer Society, are being offered to promote training and research in biometry and statistical epidemiology. Candidates who are accepted will be expected to carry out research in statistical epidemiology or the study of growth, typical or malignant.

Predoctoral fellowships: Applicants must be citizens of the United States who possess the degree of Bachelor of Arts or Bachelor of Science and must have a knowledge of biology, mathematics, and chemistry. They are expected to enter the Graduate School as candidates for the Doctor of Philosophy degree and they will be given training in one or more fields of biology

as well as statistics. These fellowships are awarded for a period of three years, but may be terminated at any time if the candidate fails to meet the standards of the University. Stipends will be $2,000 per year. Additional funds may be available for students with dependents.

Postdoctoral fellowships: These fellowships are intended for young men and women embarking upon an investigative career and also for more mature investigators desiring to extend their fields of competence. Candidates must be citizens of the United States who possess the degree of Doctor of Medicine, Doctor of Philosophy, or Doctor of Science. Fellows are expected to carry out research as indicated above and will be given training in biometry, biostatistics, and such other subjects as the University may deem necessary. Fellowships are awarded for periods of one year and may be renewed for two additional years. The stipends will range from $3,500 to $4,500 depending upon individual circumstances.

For further information, write to Professor E. Cuyler Hammond, Director of Graduate Studies in Biometry, 30 Hillhouse Ave., Yale University, New Haven, Connecticut. Applications blanks for predoctoral fellowships may be obtained from the Director of Admissions, Graduate School, Yale University, New Haven, Connecticut. All applications for both types of fellowships should be submitted as early as possible in 1956.

POST-GRADUATE COURSES IN PEDIATRICS, HARVARD MEDICAL SCHOOL (JUNE 11–22, 1956).

Two post-graduate courses in Pediatrics are being offered by the Department of Pediatrics, Harvard Medical School, at the Massachusetts General Hospital, Boston, in June 1956. The first, which will be from *June 11 through June 19*, will deal with general pediatric problems exclusive of endocrine and metabolic disorders. The second, *June 20 through June 22*, will deal specifically with parenteral fluid therapy, management of nephritis and nephrosis, diabetes and disturbances of growth, and related endocrine disorders in children and adolescents. Enrollment may be for either course or for the total combined course. Those interested should write to: Courses for Graduates, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts.